frontiers in Agronomy

ORIGINAL RESEARCH
published: 04 March 2022
doi: 10.3389/fagro.2022.805454

Plant Growth-Promoting Activity of *Bacillus* sp. PG-8 Isolated From Fermented Panchagavya and Its Effect on the Growth of *Arachis hypogea*

*Rinkal B. Gohil, Vikram H. Raval, Rakeshkumar R. Panchal and Kiransinh N. Rajput**

Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, India

A natural bacterial isolate that shows multiple plant growth-promoting activities was isolated from fermented panchagavya (a mixture of five indigenous cow products). It is a gram-positive, endospore-forming bacteria identified as *Bacillus* sp. PG-8 by 16S rRNA gene sequencing. The *Bacillus* sp. PG-8 have shown multiple plant growth-promoting activities as indole acetic acid (2.78 μg/ml), gibberellic acid (0.7 mg/ml), ammonia (6.51 μmol/ml), exopolysaccharide (2.6% w/v) production, and phosphate solubilization (198.27 μg/ml). The *Bacillus* sp. PG-8 has ability to survive under the abiotic stress conditions such as temperature (28–46°C), pH (5.0–12.0), salt (0.5–20.0% w/v NaCl), and osmotic resistance (1–10% w/v PEG-6000). Due to its diverse characteristics, the effect of *Bacillus* sp. PG-8 was tested on *Arachis hypogea* (groundnut). The seeds treated with *Bacillus* sp. PG-8 demonstrated a 70% germination rate with seedling vigor indexes of 154. In pot study, *Arachis hypogea* growth showed 1.38, 1.38, 1.32, 1.39, and 1.52 times increase in root hair number, leaf numbers, leaf width, leaf length, and leaf area, respectively. The addition of *Bacillus* sp. PG-8 culture to the *Arachis hypogea* plant resulted in a significant improvement in plant growth. *Bacillus* sp. PG-8 is a spore producer with stress tolerance and multiple plant growth-promoting properties, which makes it a potential liquid biofertilizer candidate.

Keywords: *Arachis hypogea* (Ground nut), *Bacillus* sp. PG-8, fermented panchagavya, cow products, liquid biofertilizer

OPEN ACCESS

Edited by:
Avishek Banik,
Presidency University, India

Reviewed by:
Subhrangshu Mandal,
University of Minnesota Twin Cities, United States
Sudipta Maity,
University of Rajasthan, India

****Correspondence:***
Kiransinh N. Rajput
rajputkn@gujaratuniversity.ac.in;
rajputkn@yahoo.com

Specialty section:
This article was submitted to Plant-Soil Interactions, a section of the journal Frontiers in Agronomy

Received: *30 October 2021*
Accepted: *31 January 2022*
Published: *04 March 2022*

Citation:
Gohil RB, Raval VH, Panchal RR and Rajput KN (2022) Plant Growth-Promoting Activity of Bacillus sp. PG-8 Isolated From Fermented Panchagavya and Its Effect on the Growth of Arachis hypogea. Front. Agron. 4:805454. doi: 10.3389/fagro.2022.805454

INTRODUCTION

Panchagavya refers to as "combination of five cow products of indigenous cow," which includes dung, urine, milk, curd, and ghee. Traditionally, it has been used in many ancient Indian health and sustainable agriculture customs. Fermented panchagavya is an organic liquid preparation mentioned in ancient Indian literature such as Vrikshayurveda that depicts the good farming practices for different plants (Natarajan, 2002).

Bacillus sp. is the most attractive among the plant growth-promoting rhizobacteria due to their spore-forming ability, and therefore, they contribute significantly in commercial formulations used for field application. Fermented panchagavya also contains a diverse microbial population with a 54% cultivable bacterial population dominated by the *Bacilli* group in panchagavya

preparation (Anandham et al., 2015). The bacteria isolated from panchagavya belonged to *Bacillus safensis* or *Bacillus cereus*, which showed plant growth-promoting attributes (Radha and Rao, 2014). Two plant growth-promoting bacteria (PGPB), *Bacillus pumilus* and *Bacillus subtilis,* were reported in the fermented panchagavya (Ram et al., 2019).

Biofertilizers are living microorganisms that increase the growth and also the development of plants by amassing the accessibility of mineral nutrients, solubilizing phosphorus, and production of phytohormones (Mushtaq et al., 2021). The production of plant growth regulators by microorganisms delivers several benefits to the host plant with the facilitation of root system expansion, which increases the absorption of water and nutrients and improves plant survival (Verma et al., 2017). The PGPB have some properties such as nutrient solubilization, stress tolerance, and phytohormone production (Numan et al., 2018). PGPB are colonized at or near the root of the plant, and their wave of efficacy hits the doorstep of major functional aspects of the entire plant (Mondal et al., 2020). The dominant PGPBs such as *Bacillus subtilis and Bacillus subterraneus* demonstrated considerable germination with *Arachis hypogea* in pot tests for plant growth and protection qualities (Syed et al., 2020).

The demand for pesticides and chemical fertilizers has increased enormously to get high-yield crops in contemporary agriculture practices. The current trend even upsets the delicate balance of the ecosystem. Conventional agriculture practices rely very much on the intensive use of synthetic inputs to produce the bulk of the world's food and energy crops. However, these farming systems also contribute to global climate change and environmental degradation (Crowder and Illan, 2021). Concerns over the negative impacts of conventional agriculture have spurred interest in more sustainable alternative farming systems that may replace the synthetic inputs with organic biofertilizers and pesticides (Chaudhary et al., 2017). Advantages of organic farming systems include greater profitability, increased biodiversity, enhanced soil quality, and reduced pesticides, and good quality nutritious food will lead to the overall economic development of a healthy society. The *Arachis hypogea* was used as model plant as it is a cash crop and widely sown across the state of Gujarat and many regions of India. Owing to the nutritional value and oil content of the groundnut seeds, it remains in high demand. In this context, this study provides the impact of *Bacillus* sp. PG-8 isolated from fermented panchagavya on *Arachis hypogea* growth.

MATERIALS AND METHODS

Isolation of Bacteria From Fermented Panchagavya

For the preparation of panchagavya, five cow products were collected from the Bansi Gir Gaushala in Ahmedabad, Gujarat, India. Panchagavya was a prepared method described by Gohil et al. (2020).

Fermented panchagavya samples were diluted up to 10^{-7}, and 0.1 ml of each dilution was spread onto nutrient agar plates for the isolation of bacteria. The nutrient agar plates were incubated at 30°C for 48 h. The colonies were enumerated and characteristics were recorded. Among the isolates, PG-8 bacterial isolate that shows multiple plant growth-promoting activities and survived in various abiotic stress conditions was selected for further study. The pure culture of PG-8 bacteria was maintained and preserved by periodic transfer onto nutrient agar slants at 4°C in the refrigerator.

Microbial Identification and Phylogenetic Analysis

Identification of bacterial strain PG-8 was attempted through sequence analysis of the 16S rRNA gene. Universal 16S rRNA gene primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used for amplification (Goldfarb et al., 2011). DNA sequencing reaction of PCR amplicon was carried out with BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The gene sequence was used to carry out BLAST with the database of NCBI GenBank database and aligned using multiple alignment software programs (http://www.ncbi.nlm.nih.gov/).

The evolutionary history was arranged by the neighbor-joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered organized in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). This analysis involved 9 nucleotide sequences. All ambiguous sites were removed for each sequence pairwise deletion option. Phylogenetic and molecular evolutionary analyses were conducted using MEGA 11 (Kumar et al., 2018). The nucleotide sequence of *Bacillus* sp. PG-8 was submitted to the National Centre for Biotechnology Information (NCBI).

Inoculum Preparation

The *Bacillus* sp. PG-8 culture from slant was inoculated in sterile nutrient broth and incubated at 30°C for 24 h. Cells were separated by centrifugation at 5,000 rpm for 10 min and the supernatant was removed. The cell pellet was resuspended in sterile normal saline to get optical density values of 1.0 containing $\sim 8 \times 10^8$ cells/ml. The prepared culture suspension was used as a 1.0% (v/v) inoculum to study the plant growth-promoting parameters. In the pot experiment, five ml of this culture suspension was used to treat the plants.

Plant Growth-Promoting Traits

The *Bacillus* sp. PG-8 qualitatively showed multiple plant growth-promoting activities such as the production of indole acetic acid (IAA), gibberellic acid (GA), ammonia (NH_3), exopolysaccharides (EPSs), hydrogen cyanide (HCN), and also phosphate (P) and zinc (Zn) solubilization. It was further tested to quantify the plant growth-promoting activities.

IAA Production

For IAA production, 50.0 ml of Luria-Bertani broth (pH 7.5) containing 0.1% (w/v) L-tryptophan in 250-ml flask was inoculated with 1.0% (v/v) inoculum and incubated at 30°C in

the dark, as IAA is light sensitive, 120 rpm on an orbital shaker. IAA production was carried out in 24-h intervals from 24–144 h until production was decreased.

IAA estimation

The spectrophotometric estimation of IAA was done according to Bric et al. (1991). Culture supernatant (1 ml) was mixed with 2.0 ml of Salkowski reagent and incubated at room temperature (RT) in the dark for 30 min. Development of pink color shows the production of IAA and absorbance was recorded at 530 nm. The standard calibration curve of IAA (5.0–50.0 μg/ml) was prepared to calculate the IAA production.

GA Production

Gibberellic acid production was carried out in 50.0 ml nutrient broth (pH 7.4) in 250-ml flask, 1.0% (v/v) inoculum, at 30°C, 120 rpm on an orbital shaker for 96 h. GA production was measured from 24–96 h.

GA Estimation

An equal volume of cell-free supernatant and ethyl acetate (EA) was taken in a test tube and shook vigorously. The EA extract was collected separately in a glass beaker, and the extraction was repeated three times. The separated EA extract was allowed to evaporate at room temperature. The residues were dissolved in 2.0 ml methanol. To this, 1.0 ml DNPH (2, 4 – dinitrophenylhydrazine) was added and incubated in a boiling water bath for 5 min. After incubation, it was cooled in an ice-water bath, and 5.0 ml of 10% (w/v) alcoholic potassium hydroxide was added, allowed to stand for 10 min at RT. To this, 15.0 ml of sterile distilled water was added, and the intensity of the color (red wine) produced was measured at 430 nm (Graham and Thomas, 1961). A standard calibration curve of GA (0.1–0.8 mg/ml) was prepared in methanol.

NH_3 Production

Ammonia production was determined in a test tube containing 20.0 ml sterile peptone water, pH 7.2. It was inoculated with 1.0% (v/v) inoculum and incubated at 30°C, 120 rpm on an orbital shaker for 144 h. The amount of NH_3 produced was measured every 24–144 h.

Estimation of NH_3

Spectrophotometric estimation of ammonia was done as described by Cappucino and Sherman (1992). Culture supernatant (1 ml) was mixed with 0.1 ml Nessler's reagent, and the final volume was made to 5.0 ml by adding ammonia-free distilled water. NH_3 production is indicated by a change in color from yellow to brown, and absorbance was measured at 425 nm. The NH_3 produced was calculated using the ammonium hydroxide (0.2–2.0 μmol/ml) standard calibration curve.

EPS Production

The EPS production was checked qualitatively by spot inoculation on the brain–heart infusion (BHI) agar plate, at 30°C as mucoid colonies with blackening. For quantitative EPS estimation, 100.0 ml nutrient broth (pH 7.1) containing 4.0% (w/v) sucrose was inoculated with 1.0% (v/v) inoculum and incubated at 30°C, 120 rpm on an orbital shaker up to 72 h. The amount of EPS produced was measured over 24–72 h.

Estimation of EPS

The estimation of EPS was done by solvent extraction described by Azeredo and Oliveira (1996). For extraction, 10.0 ml cell-free culture supernatant plus 30.0 ml of chilled acetone were mixed for precipitation at 4°C for 12 h. Precipitates were collected on a preweighed aluminum foil and wet weight was recorded. The precipitates were allowed to dry at 50–60°C, and the dry weight of the precipitates was recorded until three consecutive constant readings.

P Solubilization

The qualitative phosphate solubilization test was carried out using the spot inoculation technique on an agar plate containing an insoluble tricalcium phosphate (TCP) and incubated at 30°C for 120 h (Pikovskaya, 1948). A yellow zone develops surrounding the colony as a result of phosphate solubilization. The phosphate solubilization index (PSI) was calculated using the formula below.

$$PSI = \frac{Colony\ diameter + Zone\ of\ clearance}{Colony\ diameter}$$

Quantitative P Solubilization

A total of 100.0 ml sterile Pikovskaya's broth (pH 7.0 ± 0.2) in 250-ml flasks were inoculated and incubated at 30°C, at 120 rpm on an orbital shaker for 15 days. The amount of phosphate soluble was measured every 5–15 days.

The amount of phosphate solubilized by *Bacillus* sp. PG-8 was estimated by the stannous chloride method (King, 1932) and pH reduction was noted.

A total of 2.0 ml sample was centrifuged at 10,000 rpm for 20 min to separate the cells. To 0.1 ml supernatant, 0.9 ml double distilled water and 1.0 ml chloromolybdic acid was added. Contents in test tubes were diluted by adding 4.0 ml double distilled water. To this, 25.0 μL chlorostannous acid reagent was added, and the test tubes were mixed well till blue color developed. Absorbance was measured at 600 nm. The total amount of phosphate solubilized was calculated against K_2HPO_4 (10.0–100.0 μg/ml) standard calibration curve.

HCN Production

The HCN produced was detected by picrate assay with some modifications (Bakker et al., 1987). *Bacillus* sp. PG-8 was streaked on King's B agar plate supplemented with 4.4 g/L glycine. The plate was sealed with parafilm and incubated at 30°C for 168 h. HCN production was indicated a color change of filter paper from yellow to orange-brown.

Zn Solubilization

Qualitative Zn solubilization was carried out on agar plates (pH 7.0) having 0.1% (w/v) insoluble zinc oxide by spot inoculation (Bunt and Rovira, 1955). The plates were incubated at 30°C for 12 days. Zn solubilizing bacteria produce a zone of clearance around the colony.

Abiotic Stress Response of *Bacillus* sp. PG-8

The ability of *Bacillus* sp. PG-8 to tolerate different stress conditions such as salinity, drought, temperature, and pH was checked. The bacterial culture was grown on various conditions, and bacterial growth was measured on an arbitrary scale. A triple plus symbol denoted higher bacterial growth, a double plus indicate moderate growth, and a single plus indicate lower growth.

Salinity Stress

The salt tolerance of isolates was checked by observing their growth on the nutrient agar medium supplemented with different NaCl concentrations (0.5, 5, 10, 15, and 20%; w/v). The *Bacillus* sp. PG-8 was streaked on these plates and incubated at 28°C for 72 h.

Drought Stress

The drought resistance of *Bacillus* sp. PG-8 was tested on nutrient agar medium supplemented with polyethylene glycol (6000) (1, 5, 10%; w/v). The pure culture was streaked on the plates and incubated at 28°C for 72 h.

Temperature Stress

The *Bacillus* sp. PG-8 was examined for its ability to grow at 28°C, room temperature (16–33), 37, 40, and 46°C on nutrient agar media. Pure culture suspension was streaked on the plates and incubated at different temperatures for 72 h.

pH Tolerance

On nutrient agar media, pH tolerance was checked by growing *Bacillus* sp. PG-8 at different pH values 5.0, 7.0, 9.0, 11.0, and 12.0. The pure culture was streaked on the nutrient agar plates with different pH values and incubated at 28°C for 72 h.

Seed Germination Test

Seeds of *Arachis hypogea* were collected from the local agriculture seed shop, Ahmedabad, Gujarat. The seeds were washed thoroughly with water to remove the dirt followed by washing with distilled water two times to remove any remaining tap water. Then, the seeds were treated with 0.1% (v/v) of $HgCl_2$ solution for 2–3 min. Seeds were then cleaned three times with 70% (v/v) ethanol followed by rinsing with sterile distilled water five times. Disinfected seeds were used for the seed germination test and pot experiment.

Seeds (nos.100) were placed on sterile cotton in a sterile petri plate. Pure culture suspension of *Bacillus* sp. PG-8 was added to the petri plates containing seeds and incubated at room temperature. For comparative evaluation, untreated seeds were treated with distilled water used as controls. After 72 h, germinated seeds were counted, and the germination percent and seed vigor index were calculated by the following formula:

$$Germination\ percent = \frac{Seeds\ germinated}{Total\ seeds} \times 100$$

$$Seed\ vigor\ index = Seedling\ length \times Germination\ percent$$

Pot Experiment

The pot study was conducted in plastic bags with 18.0 cm × 8.0 cm height and diameter in the month of February–March, 2020, with an average temperature (35 ± 4°C) in the departmental greenhouse, Gujarat University, Ahmedabad.

About 12.0 kg soil was collected from a depth of 4.0 inches in a sterile plastic bag from the central library garden of Gujarat University Campus for a pot experiment. For the preparation of the pot, garden soil was filled with approximately 500 g of UV sterile soil having a neutral pH. The disinfected seeds of *Arachis hypogea* were sowed and watered slightly after transplanting at a depth of 0.5 cm. The experiment was carried out in duplicate for both the plants. Plants were treated with 5.0 ml *Bacillus* sp. PG-8 suspension, and the treatments were administered every five days after seeding (DAS). Two control bags were treated with sterile distilled water instead of culture. The plant from each pot was uprooted on the 10^{th}, 20^{th}, and 30^{th} day to study the effect of *Bacillus* sp. PG-8 on fresh plant weight, dry weight, shoot length, root length, stem diameter, root hair number, leaf number, leaf width, leaf length, and leaf area of individual plants.

RESULTS AND DISCUSSION

Morphological and Cultural Characteristics

A total of sixty-four culturally different bacteria were isolated from the fermented panchagavya (Gohil et al., 2020). Bacterial cultures were screened for their plant growth-promoting activities and abiotic stress tolerance. Among the isolates, a gram-positive PG-8 bacteria showed multiple plant growth-promoting activities with abiotic stress responses, and it was selected for further study. High levels of bacterial diversity and richness were reported in the fermented panchagavya (Gunwantrao and Patil, 2015). The 15 bacterial cultures were isolated from panchagavya and shown to have a variety of plant growth-promoting traits by Nagaraj and Sreenivasa (2009). A number of thirty-seven diverse groups of bacteria were isolated from panchagavya (Bhat et al., 2005). Similarly, our results confirm that fermented panchagavya is having rich bacterial diversity and many bacteria show plant growth-promoting activities.

The PG-8 is having a 4–5-mm size colony with an irregular margin and "Medusa-head" (**Figure 1**). Colonies have sticky consistency when manipulated with a loop. PG-8 is a big rod-shaped, endospore-forming bacteria that showed a gram-positive reaction (**Figure 1**). Colonial characteristics of the PG-8 are recorded in **Table 1**. The gram-positive bacterial population was found higher in panchagavya (Ram et al., 2019). The microbial load of panchagavya showed a considerable number of gram-positive bacteria (Patnaik et al., 2012). The natural bacterial isolate PG-8 from the fermented panchagavya is a gram-positive, endospore-forming, rod-shaped bacteria closely related to the *Bacillus* genus.

Identification of PG-8 Strain Through 16S RRNA Gene Sequencing

A 16S rRNA gene sequencing was done to identify PG-8. Phylogenetic analysis showed 97.4% percent similarity with the gene sequence of MT225680.1 *Bacillus haynesii*

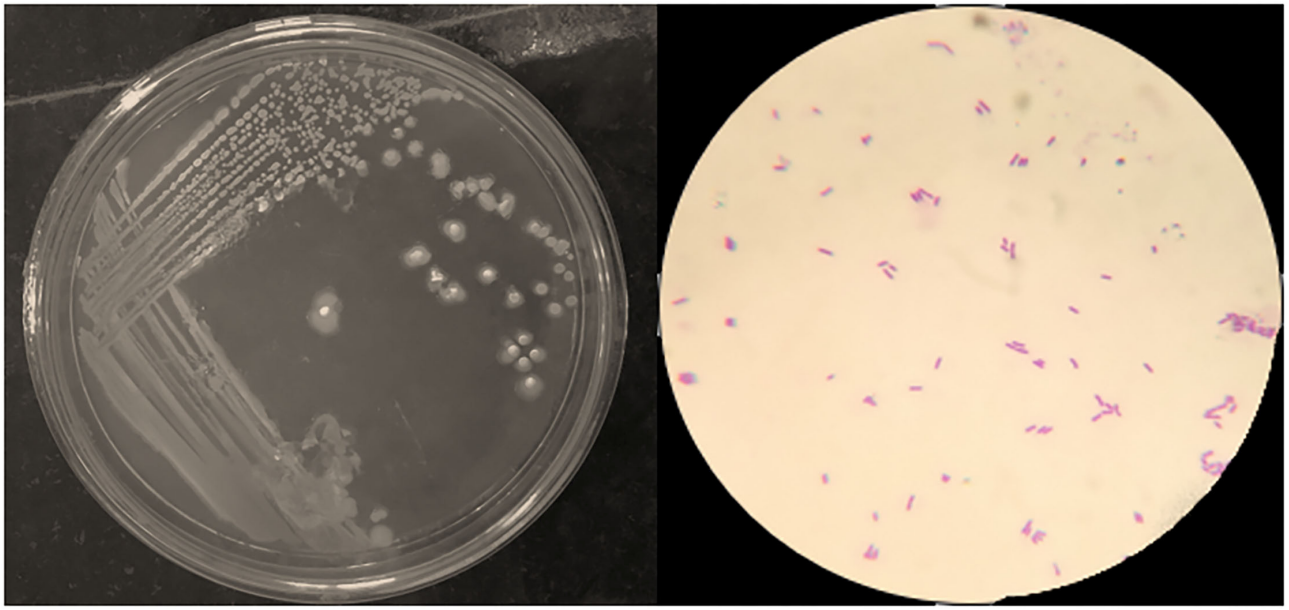

FIGURE 1 | Growth of *Bacillus* sp. PG-8 on nutrient agar plate and Gram's staining.

TABLE 1 | Colonial characteristics of *Bacillus* sp. PG-8.

Colonial characteristics and gram's reaction			
Size	Big	Margin	Entire
Shape	Irregular	Opacity	Opaque
Elevation	Slightly raised	Pigment	White
Surface texture	Smooth	Gram's reaction	Positive

strain 190311L225 MT516447.1 *Bacillus* sp. (in: Bacteria) strain L23, MT525268.1 *Bacillus paralicheniformis* strain SA25, MT527538.1 *Bacillus paralicheniformis* strain CB211, MT534566.1 *Bacillus sonorensis* strain NB35, MT534569.1:11-1370 *Bacillus licheniformis* strain NB45, MW819909.1 *Bacillus spongiae* strain DN-5, and MW876139.1 *Bacillus haynesii* strain XJB-19 that showed the highest degree of homology was used to construct a phylogenetic tree using the neighbor-joining method.

The evolutionary history was inferred using the maximum composite likelihood method (Tamura et al., 2004). The percentage of trees in which the associated taxa clustered together is shown next to the branches. The analysis involved 9 nucleotide sequences. There were a total of 1,520 positions in the final dataset. The nucleotide sequence of 1,500 base pairs *Bacillus* sp. PG-8 was deposited in the nucleotide GenBank database under the accession number OK175649.

The 16S rRNA gene sequence of the bacterial strains isolated from panchagavya belonged to *Bacillus safensis* and *Bacillus cereus* (Radha and Rao, 2014). Panchagavya contains *Bacillus aquimaris, Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Bacillus endophyticus,* and *Bacillus barbaricus* (Donaldson and Emmanuel, 2021). The bacterial strain PG-8 isolated from panchagavya has shown the phylogenetic relatedness with *Bacillus* strains (**Figure 2**). The results are in line with those reported earlier and show the potential application of genus *Bacillus* in plant growth promotion.

Primary Screening of *Bacillus* sp. PG-8 for Plant Growth-Promoting Parameters

The *Bacillus* sp. PG-8 was tested for various plant growth-promoting qualities qualitatively, which include phytostimulant production, nutrient solubilization, and plant protectants. The results revealed that the *Bacillus* sp. PG-8 possesses a variety of plant growth-promoting capabilities such as the production of IAA, GA, NH_3, EPS, and phosphate solubilization (**Table 2**).

Bacillus sp. PG-8 formed a black mucoid colony on the brain–heart infusion agar plate after 48 h, which indicates that EPS producer. Phosphate solubilization by *Bacillus* sp. PG-8 was observed in the form of a yellow zone formed around the colony on the Pikovskaya agar plate after 72 h. *Bacillus* sp. PG-8 was incapable of producing hydrogen cyanide and zinc solubilization.

Various PGPB often exhibit one or more plant growth-stimulating traits, each with distinct activity under different environmental and soil circumstances (Olanrewaju et al., 2017). PGPB have numerous plant growth-promoting characteristics such as phosphate and zinc solubilization with IAA, HCN, and exopolysaccharide production (Kalam et al., 2020). Panchagavya isolates were shown to be effective in plant growth promotion through the solubilization of insoluble phosphate and the production of indole acetic acid (Panchal, 2016). Various *Bacillus* sp. have been reported for multiple PGP activities such as IAA, GA, EPS production, and P solubilization (Bonatelli et al., 2020; Miljaković et al., 2020; Magotra et al., 2021). As PG-8 shows

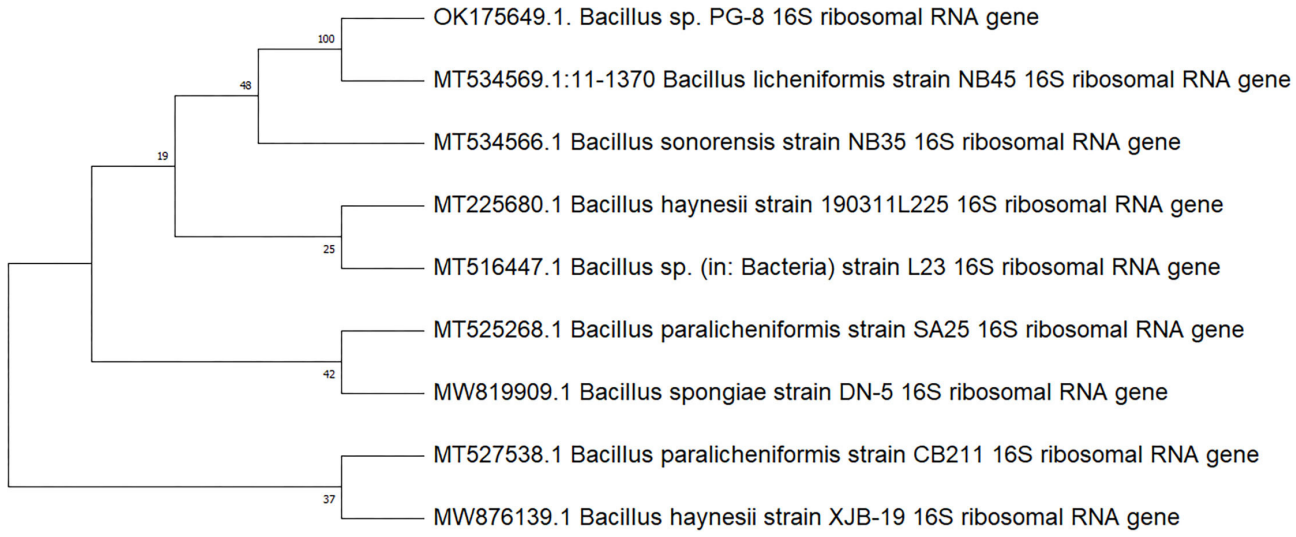

FIGURE 2 | Neighbor-joining phylogenetic tree of *Bacillus* sp. PG-8.

TABLE 2 | Primary screening of *Bacillus* sp. PG-8 for plant growth-promoting traits.

	***Bacillus* sp. PG-8**		
IAA production	Positive	PO_4 solubilization	Positive
GA production	Positive	Zn solubilization	Negative
NH_3 production	Positive	HCN production	Negative
EPS production	Positive		

multiple plant growth-promoting activities, it can be applied in the field to supplement nutrients and augment plant growth.

Secondary Screening of *Bacillus* sp. PG-8 for Plant Growth-Promoting Parameters

Indole Acetic Acid Production

Indole acetic acid production is very common among the PGPB because it helps in root expansion and uptake of nutrients. The *Bacillus* sp. PG-8 has produced 2.78 µg/ml IAA in the presence of 0.1% (w/v) tryptophan, at 30°C after 120 h (**Figure 3A**). Rhizospheric isolate *Bacillus thuringiensis* produced 0.20 µg/ml IAA with 0.1% (w/v) tryptophan after 21 h (Batista et al., 2021). *Bacillus siamensis* isolated from rhizospheric soil produced 5.14 µg/ml IAA with 0.1% (w/v) tryptophan after 72 h (Saleem et al., 2021). The *Bacillus* sp. PG-8 has reasonably produced a good amount of IAA compared to other PGPR.

Gibberellic Acid Production

Gibberellic acid is a phytohormone that helps in seed germination and plant growth. The *Bacillus* sp. PG-8 was able to produce the highest gibberellic acid 0.70 mg/ml at 30°C after 72 h in nutrient broth (**Figure 3B**). Rhizospheric isolate *Bacillus siamensis* produced 0.24 mg/ml GA after 96 h in broth (Ambawade and Pathade, 2015). *Bacillus cereus* isolated from the rhizosphere produced 0.39 mg/ml GA in a nutrient broth after 216 h (Pandya and Desai, 2013). PGPR, *Bacillus pumilus,* and *Bacillus licheniformis* produced physiologically active gibberellins in the host plant (Gutiérrez-Mañero et al., 2001). Our isolate produced double the amounts of gibberellic acid within 72 h than those reported earlier.

Ammonia Production

Ammonia production is the most common mechanism of PGPB. Considerable ammonia production was found in peptone broth by fermented panchagavya bacterial isolate. *Bacillus* sp. PG-8 produced the maximum 6.51 µmol/ml ammonia production after 120 h and was decreased after 144 h (**Figure 3C**). Agbodjato et al. (2015) reported that 80% of plant growth-promoting *Bacillus* species have shown ammonia production. Rhizobacteria, *Bacillus cereus*, and *Bacillus megaterium* produced 2.3 and 6.2 µmol/ml of ammonia, respectively, after 72 h (Dutta and Thakur, 2017). *Bacillus megaterium* isolated from soil produced 2.04 µmol/ml ammonia after 96 h (Krunal and Sanjay, 2020). Ammonia accumulation in soil may lead to alkaline conditions that suppress the growth of several plant pathogens. Production of ammonia is important for nitrogen accumulation because it promotes root, shoot, and biomass growth, which accelerates plant growth (Masclaux-Daubresse et al., 2010).

EPS Production

Bacterial exopolymer substances are released in response to the physiological stress encountered in the natural environment. Exopolysaccharide was recovered by acetone extraction, and significant production was found by panchagavya bacterial isolate. The *Bacillus* sp. PG-8 was able to produce 2.6% (w/v) EPS in nutrient broth containing 4.0% (w/v) sucrose after 24 h (**Figure 3D**). EPS production was gradually decreased then after 24 h in the broth. *Bacillus licheniformis* isolated

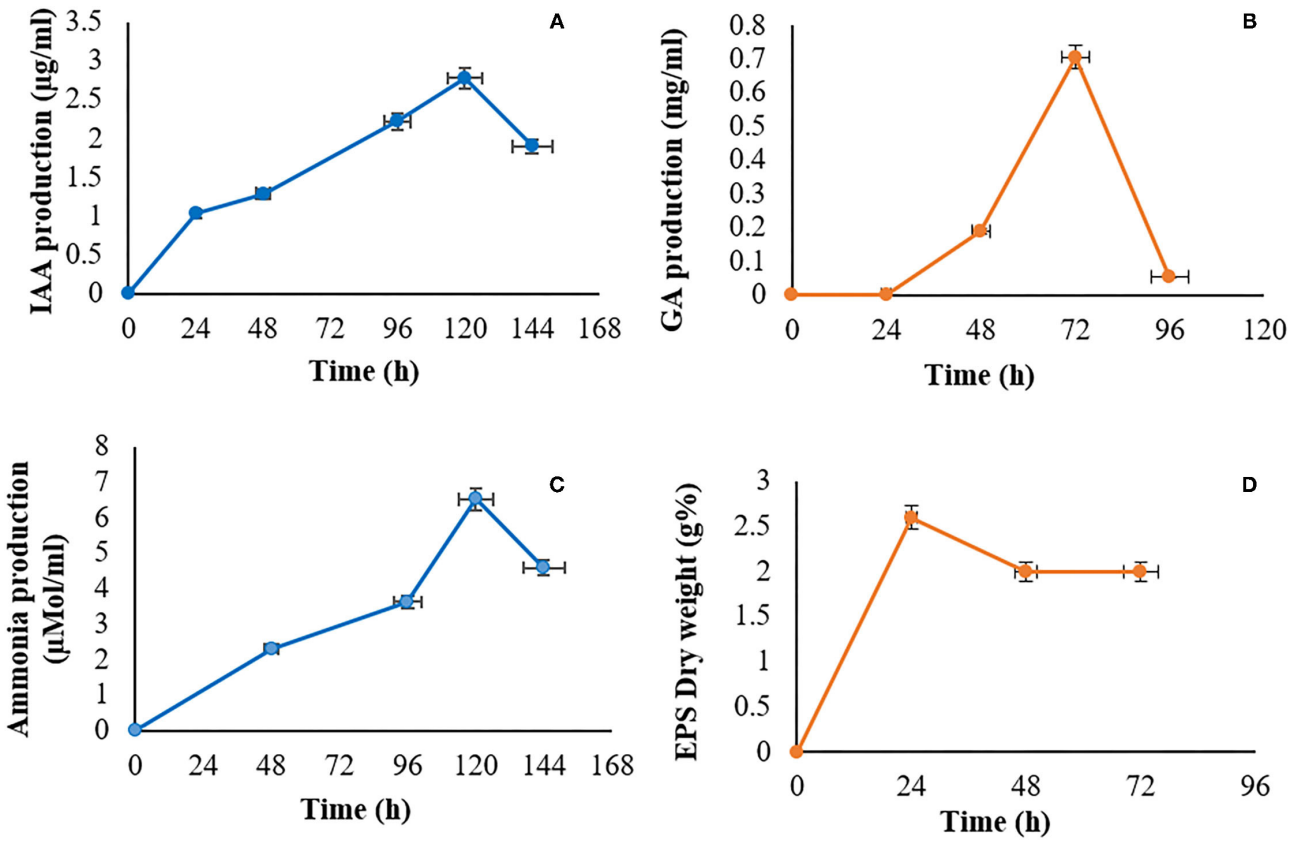

FIGURE 3 | (A) Indole acetic acid production, **(B)** Gibberellic acid production, **(C)** Ammonia production, **(D)** Exopolysaccharide production.

from soil produced 0.6% (w/v) EPS after 96 h (Gupta et al., 2021). *Bacillus licheniformis* produced 4.8% (w/v) EPS after 96 h with 10.0% (w/v) sodium succinate (Malick et al., 2017). *Paenibacillus polymyxa* isolated from wasp honeycombs was able to produce 2.4% (w/v) EPS with 5.0% (w/v) sucrose after 48 h (Liyaskina et al., 2021). The source and concentration of sugar in the medium affect EPS production. Panchagavya isolates *Bacillus* sp. PG-8 produced considerable EPS with lower sugar concentrations within 24 h. The *Bacillus* sp. PG-8 is capable of secreting EPS that may protect themselves and the host plants against abiotic stresses such as drought, salinity, or heavy metal pollution.

Phosphate Solubilization

Phosphate solubilizing bacteria improve the phosphates availability by solubilizing them and making them available to the plant. *Bacillus* sp. PG-8 has shown the phosphate solubilization index of 1.8 after 72 h. The *Bacillus* sp. PG-8 has solubilized 198 µg/ml insoluble tricalcium phosphorus after 10 days in Pikovskaya's broth (**Figure 4**). *Bacillus* sp. PG-8 was able to solubilize tricalcium phosphate sources along with a pH drop from 7.0 to 4.5 after 10 days. The *Bacillus cereus* was able to solubilize 159 µg/ml tricalcium phosphate after 10 days in Pikovskaya's broth (Salwan et al., 2021). Rhizospheric isolate *Bacillus cereus* was able to solubilize 140 µg/ml phosphate after 3 days (Abdelmoteleb and Gonzalez-Mendoza, 2020). *Bacillus xijun* isolated from rhizospheric soil solubilized 130 µg/ml phosphate after 15 days showing pH drop from 7.0 to 4.7 (Zhong et al., 2021). The *Bacillus* sp. PG-8 showed better phosphate solubilization compared to the above PGPR.

Multiple plant growth-promoting activities, such as IAA, GA, EPS, ammonia production, and P solubilization by *Bacillus* sp. PG-8 are summarized in **Table 3** with other *Bacillus* species. Panchagavya isolate *Bacillus* sp. PG-8 produced higher IAA than *B. safensis* and *B. albus*. GA production is about 30 times higher than other *Bacillus* sp. Our isolate also produced more ammonia production than *B. cereus* and showed similar phosphate solubilization.

Abiotic Stress Response

Abiotic stress is responsible for significant yield losses on crop scale worldwide. *Bacillus* sp. PG-8 responded well in abiotic stress such as, osmotic resistance, salt, temperatures, and pH and results are as noted in **Table 4**.

The growth of *Bacillus* sp. PG-8 was examined on a nutrient agar plate at temperatures ranging from 28 to 46°C, and the results revealed the growth of *Bacillus* sp. PG-8 was good over the range. *Bacillus* sp. PG-8 can survive at lower and also higher temperatures. Generally, bacterial growth is reduced at the field level in summer due to higher temperatures. Bacterial

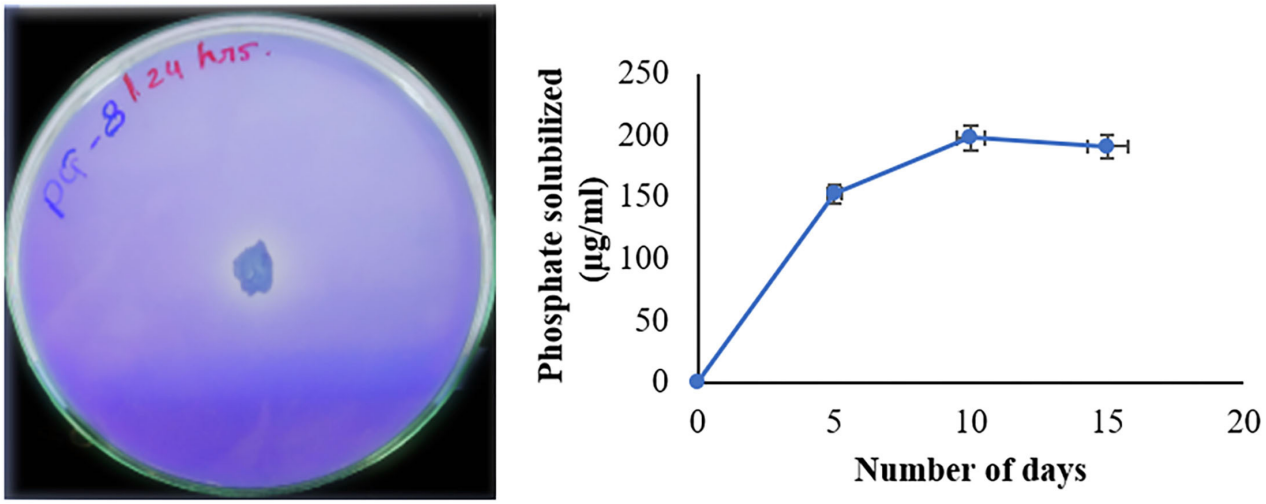

FIGURE 4 | Zone of phosphate solubilization on a plate and its rate in Pikovskaya's broth.

TABLE 3 | Plant growth-promoting traits of *Bacillus* sp. PG-8 with other *Bacillus* species.

PGPB/ Traits	IAA (µg/ml)	GA (mg/ml)	NH_3 (µmol/ml)	EPS (% w/v)	PO_4 Soluble. (µg/ml)	References
Bacillus sp. PG-8	2.78	0.700	6.51	2.600	198.0	Present study
Bacillus cereus	21.2	-	2.30	-	198.0	Dutta and Thakur, 2017
Bacillus albus	0.90	0.040	-	0.390	-	Ashry et al., 2021
Bacillus cereus	9.50	0.020	-	0.015	-	Wang et al., 2020
Bacillus safensis	0.71	0.019	-	7.300	-	Mukhtar et al., 2022

TABLE 4 | Abiotic stress response of *Bacillus* sp. PG-8.

Parameters/growth	Parameters range				
Temperature (°C)	28	RT	37	40	46
Growth	+++	+++	+++	+++	+++
Salt tolerance (% w/v)	0.5	5.0	10.0	15.0	20.0
Growth	+++	+++	+++	+	+
pH tolerance	5.0	7.0	9.0	11.0	12.0
Growth	+++	+++	+++	+++	+++
Osmotic resistance PEG-6000 (% w/v)	1.0	5.0	10.0		
Growth	+++	+++	+		

+++ = higher growth, ++ = moderate growth, + lower growth.
RT, room temperature (16–33°C).

culture's ability to withstand higher and lower temperatures is important in the preparation of liquid biofertilizers. Plants and bacteria produce several proteins that increase their tolerance to extreme temperatures. *Bacillus* sp. is more tolerant to high temperatures due to the production of heat-shock proteins and the presence of extremely resistant and dormant endospores (Getahun et al., 2020). *Bacillus cereus* and *Bacillus subtilis* can tolerate temperatures up to 45°C (Ashraf et al., 2019). *Bacillus* sp. PG-8 can be employed at the field level because of its capacity to tolerate higher temperatures.

Bacillus sp. PG-8 showed good growth on the nutrient agar plate with 0.5–10.0% (w/v) salt. The decreased growth was observed at 15 to 20% (w/v) salt but it could survive the higher salt levels. *Bacillus tequilensis* and *Bacillus aryabhattai* can tolerate up to 12% (w/v) salt (Shultana et al., 2020). The formation of biofilm and exopolysaccharides kept the viability of bacterial cells under salt stress to protect them in the rhizosphere. *Bacillus* sp. PG-8 produces more EPS, and the blackening of the colony on the BHI plate indicates that it may form a biofilm.

The growth of *Bacillus* sp. PG-8 was examined on a nutrient agar plate having different pH values (5.0, 7.0, 9.0, 11.0, and 12.0), and better growth was observed with a pH range of 5.0–12.0. The *Bacillus* sp. PG-8 may thrive in a range of pH environments, which include acidic, neutral, and basic. *Bacillus thuringiensis* was able to survive in 4.0–10.0 pH (Getahun et al., 2020). *Bacillus subtillus* growth was observed in acidic and alkaline media with a pH range of 5.0 to 8.0 on nutrient agar plates (Zahid, 2015). *Bacillus* genus has chemoreceptor adaptation systems for sensing chemical gradients (Tohidifar et al., 2020). The presence of

FIGURE 5 | Seed germination by *Bacillus* sp. PG-8.

sodium chloride, sodium carbonate, sodium hydrogen carbonate, and sulfuric acid in the soil induces crop stress (Sairam et al., 2002). PGPB may thrive in both acidic and alkaline soils and aid in the growth of plants.

The PEG (6000) at 5, 10, and 20% (w/v) concentration is equivalent to osmotic potential levels of −0.27, −0.54, and −1.09 MPa, respectively. *Bacillus* sp. PG-8 can tolerate polyethylene glycol concentrations up to 10% (w/v) and stress up to −0.54 MPa to maintain a high water activity level. *Bacillus amyloliquefaciens* and *Bacillus licheniformis* could tolerate matric stress up to −0.73 MPa (Vardharajula et al., 2011). PGPB isolate *Pseudomonas fluorescens* was able to grow in stress at a minimum water potential (-0.30 MPa) with PEG-6000 (Niu et al., 2018). *Bacillus amyloliquefaciens* has the ability to sustain pepper growth in the face of drought, salt, and heavy metal stress (Kazerooni et al., 2021). *Bacillus thuringiensis* showed better temperature tolerance at 45°C, tolerance of acidic and alkaline pH, high salinity, and drought resistance (Getahun et al., 2020). Significant effects of plant tolerance to abiotic stress are that it will result in promoting the yield and production of crops to feed humans and livestock. *Bacillus* sp. PG-8 showed that multiple abiotic stress tolerance hence could be useful for field application.

Seed Germination

Seed germination is one of the most crucial phases of the growth cycle in plant development. The percentage germination ability of *Bacillus* sp. PG-8 is shown in **Figure 5**. The seeds of *Arachis hypogea* treated with *Bacillus* sp. PG-8 showed 75 percent seed germination with 154 seed vigor index whereas control showed 70 percent and 110, respectively. *Bacillus licheniformis* culture-treated *Arachis hypogea* seedlings showed 85% germination (Shifa et al., 2015). Seed germination is a parameter of prime significance and fundamental to total biomass and yield production. As *Bacillus* sp. PG-8 has a beneficial effect on *Arachis hypogea* seed germination, it can be used to promote plant growth in the field.

TABLE 5 | Effect of *Bacillus* sp. PG-8 on the growth of *Arachis hypogea*.

Physico vegetative parameters	Treatment (days)					
	10	20	30	10	20	30
	Control			Treated		
Fresh weight (g)	0.25	0.32	0.35	0.26	0.33	0.38
Dry weight (g)	0.22	0.28	0.32	0.21	0.27	0.37
Shoot length (cm)	5.01	17.0	22.0	5.50	19.0	24.0
Root length (cm)	2.10	2.20	3.50	3.10	3.21	3.80
Root hair number	6.00	10.0	18.0	11.0	19.0	25.0
Leaf width (cm)	1.11	1.42	1.55	1.22	1.40	1.90
Leaf length (cm)	1.02	1.60	2.80	1.20	1.81	3.90
Leaf number	12.0	20.0	26.0	15.0	29.0	36.0
Leaf area (cm^2)	1.10	2.24	5.04	1.44	2.52	7.70
Stem diameter (mm)	1.22	1.54	1.71	1.52	1.65	1.82

Pot Experiment

In primary tests, *Bacillus* sp. PG-8 was tested for its beneficial role in producing phytohormones and releasing phosphorus in the broth medium. The biological contribution of *Bacillus* sp. PG-8 on the growth of *Arachis hypogea* was investigated, and the results were recorded with control (**Table 5**). The results revealed that the pure culture of

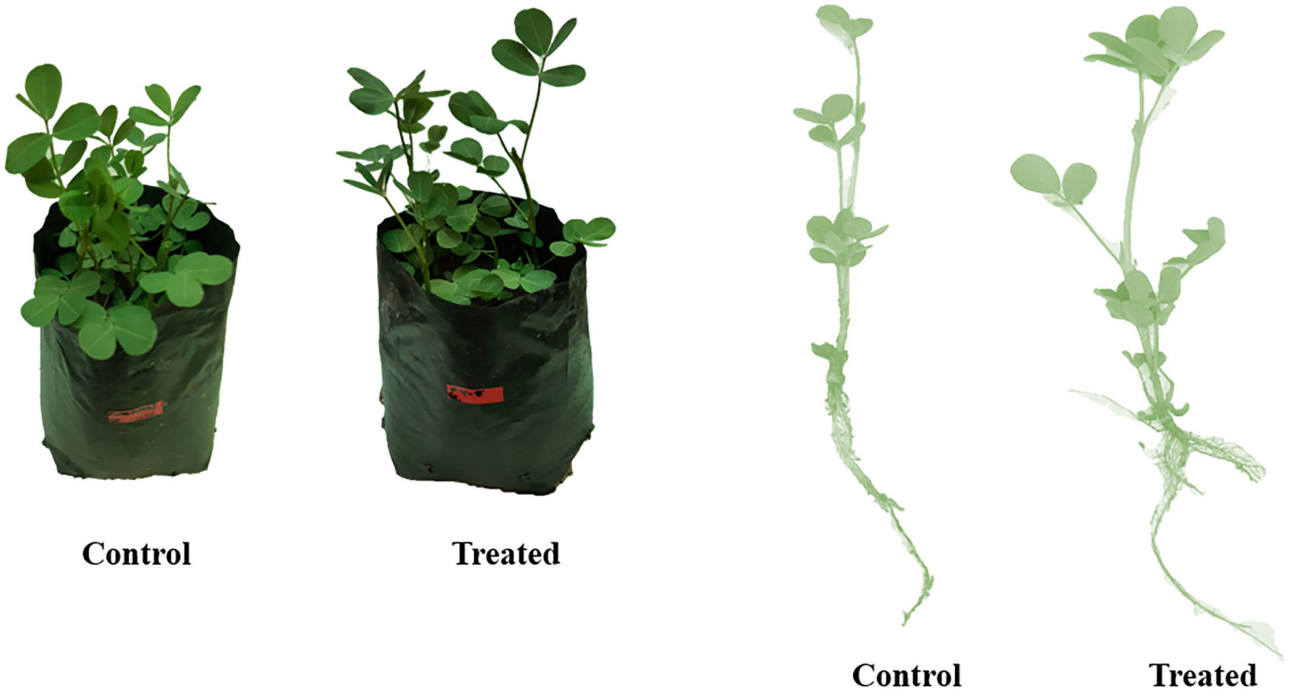

FIGURE 6 | *Arachis hypogea* plant after 30 days.

Bacillus sp. PG-8 has a great influence on the development of the plant.

It was observed that treated plants had longer roots and shoots, more number of leaves with better breadth and length than the untreated control plants (**Figure 6**). In addition, treated plants had a higher fresh weight and dry weight. *Arachis hypogea* inoculated with *Bacillus* sp. PG-8 showed remarkable improvements in the fresh weight, dry weight, shoot length, root length, root hair number, and stem diameter by 1.08, 1.15, 1.09, 1.08, 1.38, and 1.05 times, respectively, after the 30th day. *Arachis hypogea* inoculated with *Bacillus* sp. PG-8 showed remarkable improvements in the leaf numbers, leaf width, leaf length, and leaf area by 1.38, 1.32, 1.39, and 1.52 times, respectively, after the 30th day (**Figure 6**).

Bacillus species, such as *Bacillus subtilis, Bacillus amyloliquoefaciens, Bacillus cereus, Bacillus pumilus, and Bacillus polymyxa,* are well known for their plant growth and development abilities because they produce antibiotics and produce phytohormones and phosphate solubilization (Majumdar, 2017). The *Bacillus* sp. PG-8-treated plants showed growth parameters that indicated that they can survive easily in soil. The *Bacillus* sp. was able to solubilize the additional insoluble nutrients and simultaneously help the plant growth. *Bacillus* is the most common nonrhizobial endophytic genus in summer crops, and it can enhance plant root development (Bhutani et al., 2018). By regulating auxin-responsive genes, PGPR regulates endogenous IAA levels in rice roots, which causes root architectural alterations (Ambreetha et al., 2018). *Bacillus* sp. was effectively exploited to enhance the growth and yield of *V. radiata. Bacillus megaterium, Bacillus pumilus*, and *Bacillus subtilis* increased shoot length by 55.55, 46.46, and 46.20% over control, respectively (Kumari et al., 2018). *Arachis hypogea* treated with *Bacillus licheniformis* exhibited good plant development (Shifa et al., 2015). Inoculation of soil or crops with PGPB is a potential technique for boosting plant growth, thus reducing the usage of environmentally harmful chemical fertilizers (Alori et al., 2012). The increase in growth attributes of *Arachis hypogea* might be due to the involvement of *Bacillus* sp. PG-8 by increasing the availability of nutrients, biosynthesis of plant growth regulators such as auxins, gibberellic acid, and phosphate solubilization.

CONCLUSION

In this study, PGPB was isolated from fermented panchagavya. The *Bacillus* sp. PG-8 has multiple plant growth-promoting properties such as the production of gibberellic acid, indole acetic acid, ammonia, exopolysaccharide, and phosphate solubilization. Application of *Bacillus* sp. PG-8 improved *Arachis hypogea* growth as compared to control. It showed considerable improvements in the shoot length, root length, root hair number, leaf number, and leaf area in both plants. A spore-forming *Bacillus* sp. PG-8 shows multiple plant growth-promoting abilities and can survive in stress conditions, which makes it a potential candidate for application as bioinoculants and biofertilizers.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

RG: concept, experimental work, and manuscript writing. VR: data curation and review and editing. RP: language editing, data curation, and manuscript shaping. KR: conceptualization, data curation, supervision, writing original draft, review and editing. RG, VR, RP, and KR: contributed to manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

Infrastructure facility of the Department of Microbiology and Biotechnology, Gujarat University is duly acknowledged.

REFERENCES

Abdelmoteleb, A., and Gonzalez-Mendoza, D. (2020). Isolation and identification of phosphate solubilizing *Bacillus* sp. from *Tamarix ramosissima* rhizosphere and their effect on the growth of *Phaseolus vulgaris* under salinity stress. *Geomicrobiol. J*. 37, 901–908. doi: 10.1080/01490451.2020.1795321

Agbodjato, N. A., Noumavo, P. A., Baba-Moussa, F., Salami, H. A., Sina, H., Sèzan, A., et al. (2015). Characterization of potential plant growth-promoting rhizobacteria isolated from Maize (*Zea mays L*.) in central and Northern Benin (West Africa). *Appl. Environ. Soil Sci*. 2015. doi: 10.1155/2015/901656

Alori, E. T., Fawole, O., and Afolayan, A. (2012). Characterization of arbuscular mycorrhizal spores isolated from Southern Guinea Savanna of Nigeria. J. Agric. Sci. 4(7), 13-19. doi: 10.5539/jas.v4n7p13

Ambawade, M. S., and Pathade, G. R. (2015). Production of gibberellic acid by *Bacillus siamensis* BE 76 isolated from banana plant (Musa sp.). *Int. J. Sci. Res*. 4, 394–398. Available online at: https://www.ijsr.net/archive/v4i7/SUB152006.pdf

Ambreetha, S., Chinnadurai, C., Marimuthu, P., and Balachandar, D. (2018). Plant-associated *Bacillus* modulates the expression of auxin-responsive genes of rice and modifies the root architecture. *Rhizo*. 5, 57–66. doi: 10.1016/j.rhisph.2017.12.001

Anandham, R., Premalatha, N., Jee, H. J., Weon, H. Y., Kwon, S. W., Krishnamoorthy, R., et al. (2015). Cultivable bacterial diversity and early plant growth promotion by the traditional organic formulations prepared using organic waste materials. *Int. J. Recycl. Org. Waste Agric*. 4, 279–289. doi: 10.1007/s40093-015-0107-1

Ashraf, A., Bano, A., and Ali, S. A. (2019). Characterisation of plant growth-promoting rhizobacteria from rhizosphere soil of heat-stressed and unstressed wheat and their use as bio-inoculant. *J. Plant Biol*. 21, 762–769. doi: 10.1111/plb.12972

Ashry, N. M., Alaidaroos, B. A., Mohamed, S. A., Badr, O. A., El-Saadony, M. T., and Esmael, A. (2021). Utilization of drought-tolerant bacterial strains isolated from harsh soils as a plant growth-promoting rhizobacteria (PGPR). ***Saudi J. Biol. Sci***. doi: 10.1016/j.sjbs.2021.10.054

Azeredo, J., and Oliveira, R. (1996). A new method for precipitating bacterial exopolysaccharides. *Biotech. Tech*. 10, 341–344. doi: 10.1007/BF00173251

Bakker, A. W., Cosse, A. A., and Schippers, B. (1987). The role of HCN-producing Pseudomonas sp. in yield reductions in short potato rotations. Mededelingen van de Faculteit landbouwwetenschappen. *Rijksuniversiteit Gent*. 52, 1111–1117.

Batista, B. D., Dourado, M. N., Figueredo, E. F., Hortencio, R. O., Marques, J. P. R., Piotto, F. A., et al. (2021). The auxin-producing *Bacillus thuringiensis* RZ2MS9 promotes the growth and modifies the root architecture of tomato (*Solanum lycopersicum* cv. Micro-Tom). *Arch. Microbiol*. 1, 3869–3882. doi: 10.1007/s00203-021-02361-z

Bhat, S. S., Vinu, A. K., and Naidu, R. (2005). Association of diverse groups of bacteria with 'Panchagavya' and their effect on growth promotion of coffee seedlings. In: *ASIC 2004. 20th International Conference on Coffee Science, Bangalore, India, 11-15 October 2004*. Association Scientifique Internationale du Café (ASIC) p. 1192–1198. Available online at: https://www.tib.eu/en/search/id/BLCP:CN061085152

Bhutani, N., Maheshwari, R., Negi, M., and Suneja, P. (2018). Optimization of IAA production by endophytic *Bacillus* sp. from *Vigna radiata* for their potential use as plant growth promoters. *Isr. J. Plant Sci*. 65, 83–96. doi: 10.1163/22238980-00001025

Bonatelli, M. L., Li, J., Dumaresq, A., Quecine, M. C., and A. N. D., Settles, M. L. (2020). Complete genome sequence of *Bacillus* sp. Strain RZ2MS9, a multitrait plant growth promoter. *Microbiol. Resour. Announc*. 9, e00623–20. doi: 10.1128/MRA.00623-20

Bric, J. M., Bostock, R. M., and Silverstone, S. E. (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol*. 57, 535–538. 10.1128/aem.57.2.535-538.1991. doi: 10.1128/aem.57.2.535-538.1991

Bunt, J. S., and Rovira, A. D. (1955). Microbiological studies of some subantarctic soils. *J. of Soil Sci*. 6, 119–128. doi: 10.1111/j.1365-2389.1955.tb00836.x

Cappucino, J. C., and Sherman, N. (1992). *Nitrogen Cycle. Microbiology: A Laboratory Manual, 3rd Edn*. New York, NY: Benjamin/Cumming Publications.

Chaudhary, S., Kanwar, R. K., Sehgal, A., Cahill, D. M., Barrow, C. J., Sehgal, R., et al. (2017). Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Fron. Plant Sci*. 8, 610. doi: 10.3389/fpls.2017.00610

Crowder, D. W., and Illan, J. G. (2021). Expansion of organic agriculture. *Nature Food*. *2*, 324–325. doi: 10.1038/s43016-021-00288-8

Donaldson, E., and Emmanuel, C. J. (2021). *Isolation and Identification of Bacillus Species Present in the Panchagavya Preparations. Undergraduate Research Symposium (URS 2021)*. Physics Society, University of Jaffna. Available online at: http://repo.lib.jfn.ac.lk/ujrr/handle/123456789/1457

Dutta, J., and Thakur, D. (2017). Evaluation of multifarious plant growth-promoting traits, antagonistic potential and phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling, India. *PloS One*. 12, e0182302. doi: 10.1371/journal.pone.0182302

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evol*. 39:83–791. doi: 10.1111/j.1558-5646.1985.tb00420.x

Getahun, A., Muleta, D., Assefa, F., and Kiros, S. (2020). Plant Growth-Promoting Rhizobacteria Isolated from Degraded Habitat Enhance Drought Tolerance of Acacia (*Acacia abyssinica Hochst. ex Benth*.) *Seedlings. Int J Microbiol*. 10.1155/2020/8897998. doi: 10.1155/2020/8897998

Gohil, R., Patel, K., Shah, H., Vyas, S., Desai, M., and Rajput, K. (2020). Isolation and Screening of Plant Growth Promoting Bacteria from Fermented Panchagavya. *Biosci. Biotechnol. Res. Commun*. 13, 42–48. doi: 10.21786/bbrc/13/1

Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., et al. (2011). Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Front. Microbio*. 2, 94. doi: 10.3389/fmicb.2011.00094

Graham, H. D., and Thomas, L. B. (1961). Rapid, Simple Colorimetric Method for the Determination of Micro Quantities of Gibberellic Acid. *J. Pharmaceu. Sci*. 50, 44–48. doi: 10.1002/jps.2600500110

Gunwantrao, B. B., and Patil, C. R. (2015). Bacterial succession during Panchagavya making as revealed by DGGE analysis. *J Pure Appl Microbiol*. 9, 3005–3014.

Gupta, J., Rathour, R., Dupont, C. L., Kaul, D., and Thakur, I. S. (2021). Genomic insights into waste valorized extracellular polymeric substances (EPS) produced by *Bacillus* sp. ISTL8. Environ. Res., 192, 110277. doi: 10.1016/j.envres.2020.110277

Gutiérrez-Mañero, F. J., Ramos-Solano, B., Probanza, A. N., Mehouachi, J., R., Tadeo, F., et al. (2001). The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol. Plant*. 111, 206–211. doi: 10.1034/j.1399-3054.2001.1110211.x

Kalam, S., Basu, A., and Podile, A. R. (2020). Functional and molecular characterization of plant growth promoting *Bacillus* isolates from tomato rhizosphere. *Heliyon*. 6, e04734. doi: 10.1016/j.heliyon.2020.e04734

Kazerooni, E. A., Maharachchikumbura, S. S., Adhikari, A., Al-Sadi, A. M., Kang, S. M., Kim, L. R., et al. (2021). Rhizospheric *Bacillus amyloliquefaciens* protects *Capsicum annuum* cv. *Geumsugangsan* from multiple abiotic stresses via multifarious plant growth-promoting attributes. *Front. Plant Sci*. 12, 821. doi: 10.3389/fpls.2021.669693

King, E. J. (1932). The colorimetric determination of phosphorus. *Biochem*. 26, 292–297. 10.1042/bj0260292. doi: 10.1042/bj0260292

Krunal, M., and Sanjay, J. (2020). *Bacilli* consortia positively regulates agronomic and growth traits in rice. *Int. J. Chem. Stud*. 8, 636–645. doi: 10.22271/chemi.2020.v8.i3h.9278

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mole. Biol. Evolu*. 35:1547–1549. doi: 10.1093/molbev/msy096

Kumari, P., Meena, M., Gupta, P., Dubey, M. K., Nath, G., and Upadhyay, R. (2018). Plant growth promoting rhizobacteria and their biopriming for growth promotion in mung bean (*Vigna radiata* (L.) R. Wilczek). *Biocatal. Agric. Biotechnol*. 16, 163–171. doi: 10.1016/j.bcab.2018.07.030

Liyaskina, E. V., Rakova, N. A., Kitykina, A. A., Rusyaeva, V. V., Toukach, P. V., Fomenkov, A., et al. (2021). Production and characterization of the exopolysaccharide from strain *PaeniBacillus polymyxa* 2020. *PloS ONE*. 16, e0253482. doi: 10.1371/journal.pone.0253482

Magotra, S., Bhagat, N., Ambardar, S., Ali, T., Hurek, B. R., Hurek, T., et al. (2021). Field evaluation of PGP *Bacillus* sp. strain D5 native to Crocus sativus, in traditional and non traditional areas, and mining of PGP genes from its genome. *Scientific Reports*. 11, 1–16. doi: 10.1038/s41598-021-84585-z

Majumdar, S. (2017). *Evaluation of jute rhizospheric bacteria for plant growth promotion and disease suppression* (Doctoral dissertation), University of North Bengal).

Malick, A., Khodaei, N., Benkerroum, N., and Karboune, S. (2017). Production of exopolysaccharides by selected *Bacillus* strains: Optimization of media composition to maximize the yield and structural characterization. *Int. J. Biol. Macromol*. 102, 539–549. 0.1016/j.ijbiomac.2017.03.151. doi: 10.1016/j.ijbiomac.2017.03.151

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., and Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals Bot*. 105, 1141–57. doi: 10.1093/aob/mcq028

Miljaković, D., Marinković, J., and Balešević-Tubić, S. (2020). The significance of *Bacillus* sp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*. 8, 1037. doi: 10.3390/microorganisms8071037

Mondal, S., Halder, S. K., Yadav, A. N., and Mondal, K. C. (2020). Microbial consortium with multifunctional plant growth-promoting attributes: future perspective in agriculture. *Advances in Plant Microbiome and Sustainable Agriculture*. 219–258. doi: 10.1007/978-981-15-3204-7_10

Mukhtar, T., Ali, F., Rafique, M., Ali, J., Afridi, M. S., Smith, D., et al. (2022). Biochemical characterization and potential of *Bacillus safensis* strain SCAL1 to mitigate heat stress in *Solanum lycopersicum L. J. Plant Growth Regul*. 1–16. doi: 10.1007/s00344-021-10571-4

Mushtaq, Z., Faizan, S., and Hussain, A. (2021). "Role of Microorganisms as Biofertilizers", in *Microbio. and Bioferti*. Cham: Springer. p. 83–98. doi: 10.1007/978-3-030-48771-3_6

Nagaraj, N., and Sreenivasa, M. N. (2009). Influence of bacteria isolated from panchagavya on seed germination and seed vigour in wheat. *Karnataka J. of Agri. Sci*. 22, 231–232.

Natarajan, K. (2002). *Book on Panchagavya – Panchagavya Manual*. Mapusa, Goa, India: Other India Press.

Niu, X., Song, L., Xiao, Y., and Ge, W. (2018). Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. *Front. Microbiol*. 8, 2580. doi: 10.3389/fmicb.2017.02580

Numan, M., Bashir, S., Khan, Y., Mumtaz, R., Shinwari, Z. K., Khan, A. L., et al. (2018). Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiol. Res*. 209, 21–32. doi: 10.1016/j.micres.2018.02.003

Olanrewaju, O. S., Glick, B. R., and Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World J. Microbiol. Biotechnol*. 33, 1–16. 10.1007/s11274-017-2364-9. doi: 10.1007/s11274-017-2364-9

Panchal, V. V. (2016). *Agriculturally beneficial bacterial isolates from panchagavya and establishing their PGP role in chilli (Capsicum annuum L.)* (Doctoral dissertation). Anand: Anand Agricultural University).

Pandya, N., and Desai, P. (2013). Gibberellic Acid Production by *Bacillus cereus* Isolated from the Rhizosphere of Sugarcane. *J. Pure Appl. Microbiol.* 7, 3239–3242.

Patnaik, H. P., Dash, S. K., and Shailaja, B. (2012). Microbial composition of Panchagavya. *J. Eco. Agri.* 7, 101–103.

Pikovskaya, R. I. (1948). *Mobilization of phosphorus in soil in connection with the vital activity of some microbial species*. Available online at: https://ci.nii.ac.jp/naid/10026513896/

Radha, T. K., and Rao, D. L. N. (2014). Plant growth promoting bacteria from cow dung based biodynamic preparations. *Indian J. Microbiol*. 54, 413–418. doi: 10.1007/s12088-014-0468-6

Ram, R. A., Ahmad, I., and Kumar, A. (2019). Isolation and characterization of PGPRs from organic preparations. *J. Eco. Agricul*. 14, 18–22. Available online at: http://krishi.icar.gov.in/jspui/handle/123456789/55317

Sairam, R. K., Rao, K. V., and Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci. 163*, 1037–1046. doi: 10.1016/S0168-9452(02)00278-9

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol.Evol.* 4, 406–425. 10.1093/oxfordjournals.molbev.a040454.

Saleem, S., Iqbal, A., Ahmed, F., and Ahmad, M. (2021). Phytobeneficial and salt stress mitigating efficacy of IAA producing Salt Tolerant strains in *Gossypium hirsutum*. *Saudi J. Biol. Sci.* 10.1016/j.sjbs.2021.05.056. doi: 10.1016/j.sjbs.2021.05.056

Salwan, R., Sharma, V., Saini, R., and Pandey, M. (2021). Identification of plant beneficial *Bacillus* sp. for Resilient agricultural ecosystem. *Cur. Res. in Micro. Sci*. 2, 100046. doi: 10.1016/j.crmicr.2021.100046

Shifa, H., Gopalakrishnan, C., and Velazhahan, R. (2015). Efficacy of *Bacillus subtilis* G-1 in suppression of stem rot caused by *Sclerotium rolfsii* and growth promotion of groundnut. *Int. J. Environ.* 8, 111–118. doi: 10.5958/2230-732X.2015.00015.7

Shultana, R., Kee Zuan, A. T., Yusop, M. R., and Saud, H. M. (2020). Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice. *Plos ONE*. 15, e0238537. doi: 10.1371/journal.pone.0238537

Syed, S., Tollamadugu, N. P., and Lian, B. (2020). *Aspergillus* and *Fusarium* control in the early stages of *Arachis hypogaea* (groundnut crop) by plant growth-promoting rhizobacteria (PGPR) consortium. *Microbiol. Res*. 240, 126562. doi: 10.1016/j.micres.2020.126562

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. U.S.A (USA)*. 101, 11030–11035. doi: 10.1073/pnas.0404206101

Tohidifar, P., Plutz, M. J., Ordal, G. W., and Rao, C. V. (2020). The mechanism of bidirectional pH taxis in *Bacillus* subtilis. *J. Bacteriol*. 202, e00491–19. 10.1128/J*Bacillus*00491-19. doi: 10.1128/JB.00491-19

Vardharajula, S., Zulfikar Ali, S., Grover, M., Reddy, G., and Bandi, V. (2011). Drought-tolerant plant growth promoting *Bacillus* sp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interact*. 6, 1–14. doi: 10.1080/17429145.2010.535178

Verma, P., Yadav, A. N., Kumar, V., Singh, D. P., and Saxena, A. K. (2017). Beneficial plant-microbes interactions: biodiversity of microbes from diverse extreme environments and its impact for crop improvement. *Plant-Micro. Interct. Agro-eco. Perspe. Springer, Singapore*. 543–580. doi: 10.1007/978-981-10-6593-4_22

Wang, X., Xie, H., Ku, Y., Yang, X., Chen, Y., Yang, N., et al. (2020). Chemotaxis of *Bacillus cereus* YL6 and its colonization of Chinese cabbage seedlings. *Plant Soil*. 447, 413–430. doi: 10.1007/s11104-019-04344-y

Zahid, M. (2015). Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). *Front. Microbiol.* 6, 207. doi: 10.3389/fmicb.2015.00207

Zhong, J., Hu, X., Liu, X., Cui, X., Lv, Y., Tang, C., et al. (2021). Isolation and identification of uranium tolerant phosphate-solubilizing *Bacillus* sp. and their synergistic strategies to U (VI) immobilization. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.676391

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.