GAU Research Journal **49** (2) : 197-207 (July 2024)

Plant growth promoting activities of bacteria isolated form rhizospheric soil and their impact on *Vigna Radiata* L.

Krunal Hirpara[1], Bhavdeep Padsala[1], Charmy Kothari[2], Ramesh Kothari[1*]

[1]Department of Biosciences, Saurashtra University, Rajkot 360005, India
[2]Department of Biotechnology, Christ College, Rajkot 360005, India
*Email : kothari1971@gmail.com

Received : April, 2024 Accepted : July, 2024

ABSTRACT

Rhizospheric microbial communities play a vital role in plant nutrition, health, and soil fertility. Four bacterial isolates (B-1, B-6, B-10, and B-11) obtained from rhizospheric soil were evaluated for their plant growth-promoting traits, including nitrogen fixation, phosphate and potassium solubilization, siderophore production, and indole acetic acid (IAA) production. All isolates demonstrated the ability to fix nitrogen. Further, B-11 showed the highest phosphate (8.42) and potassium (27.66) solubilization index, while B-1 produced maximum siderophores. B-10 recorded the highest IAA production (4.5 µg/ml) without L-tryptophan, whereas B-11 showedmore IAA production (22 µg/ml) in the presence of L-tryptophan. Application of pure cultures significantly enhanced plant growth parameters of *Vigna radiata* L. Furthermore, seeds treated with B-11 displayed notable increases in fresh weight (76.43%), total length (29.41%), shoot length (21.43%), root length (39.13%), number of root branches (400%), number of leaves (37.50%), and dry weight (71.43%) after 30th days. These findings highlight B-11 as a promising bioinoculant for sustainable crop improvement.

Keywords : Nitrogen fixation, Solubilization, Siderophore production, Indole acetic acid, *Vigna radiata* L.

INTRODUCTION

The area of soil where the root system most impacts microorganism-mediated activities is known as the rhizosphere. This region, which includes the soil near the plant roots and frequently stretches a few millimeters beyond the root surface, is crucial for the interactions between plants and microorganisms (Gray and Smith, 2005). This is because plant roots release a wide range of molecular compounds that attract organisms that may be advantageous to plants or neutral to them (Badri and Vivanco, 2009).

Plant growth-promoting bacteria, also known as PGPBs, are a wide-ranging and helpful group of microbes present in the rhizosphere, on the surface of the roots, or close to them. They can protect plants from abiotic stresses and disease while promoting plant growth (Dimkpa et al., 2009a; Grover et al., 2011; Glick, 2012). PGPB stimulates growth of plants and biocontrol through various indirect and direct methods. PGPB have been used as a new technique in modern agriculture to

increase crop growth and yield in a sustainable manner (Naveed et al., 2008). Individual or consortium of microorganism inoculants have been provento drastically boost crop yields at a fraction of the price of chemical fertilizers. Many studies have been conducted to confirm this promising practice (Efthimiadou et al., 2020; Drobek et al., 2019).

The solubilization of phosphate and potassium, nitrogen fixation, iron acquisition via siderophore, and modulation of appropriate levels of plant hormones such as cytokinins, auxinsand gibberellins, as well as the reduction of ethylene levels through the synthesis of the ACC deaminase enzyme, are some of the direct mechanisms of PGPB (Mushtaq et al., 2021). Among the indirect processes of PGPBis the production of several enzymes and chemicals, such as chitinase, protease, cellulase, antibiotics, HCN, ammonia, and volatile organic compounds (VOCs), which in turn reduce fungal, bacterial, and nematode pathogens.

Soil is the main source of nutrients that plants require for development. Nitrogen (N), phosphorus (P) and potassium (K) are the three primary nutrients. Nitrogen is anessential element for plant growth and development. It is the most abundant element in the atmosphere, but plants cannot use it biochemically. Through biological nitrogen fixation processes, PGPB fixed atmospheric N_2 into ammonia, nitrite, or related nitrogenous compounds in soil. Phosphorous (P) is the second most common mineral nutrient, often limiting the growth development of crops (Azziz et al., 2012). Phosphorous (P) is second most important mineral after nitrogen nutrients, most frequently limiting the growth of crops. On an average, approximately 0.05% (w/w) phosphorus content was found in soil, out of which only 0.1% phosphorus form (soluble form) is available for plant absorption. Phosphate and potassium solubilizing bacteria increase phosphorus and potassium availability for plant use by solubilizing insoluble soil P and K (Hii et al., 2020).

The host plant gains various advantages from the bacterial synthesis of growth regulators, including encouraging root system development, which enhances nutrition and water absorption and promotes plant durability (Verma et al., 2017). Thickened cell walls, cuticles, and structural alterations to roots, stems, and leaves are examples of phytohormones. Therefore, the goal of the current effort was to isolate, screen, and characterize the PGPB from rhizospheric soils which might be used in the future to get different plant products in sustainable agricultural manner.

MATERIAL AND METHODS

Bacterial isolates

Aagricultural rhizospheric soil sample was collected from a *Solanum tuberosum*L. (potato) crop in Sikkim to isolate plant growth-promoting bacteria.Isolation was done onthree selective media (Jensen's medium, Pikovskaya's medium and Aleksandrow medium) using 10^{-4} and 10^{-5} dilution and incubated at 28°C and 38°C for 120 h in duplicates. All isolates were maintained at 4°C in the refrigerator by transferring onto Nutrient agar (N-agar) plates. The bacterial morphology of all bacterial cultures was recorded after Gram's staining. The colonial characteristics of all bacteria were noted from the well-isolated bacterial colony grown on a N-agar plate after

48 h. Among the isolates, four bacterial isolates (B-1, B-6, B-10 and B-11) were selected for further study because of their multiple plant growth-promoting characteristics.

Preservation of isolates

The pure cultures of bacterial isolates were grown on N-agar medium, and colony were transferred to glycerol stock for long-term preservation at -20°C.

Inoculum preparation

In order to prepare the inoculum, 30ml of N-broth was inoculated with selectedbacterial cultures from N-agar and incubated at 28°C,120 rpm for 24 h. Furthermore, the bacterial cellswere separated by centrifugation at 8,000 rpm for 10 minutes, andcollected cells were resuspended in normal saline water in a way that cell density of selected bacteria was maintained ~ 10^8 cells/ml, which was used to study the different PGP activity.

Plant growth-promoting characteristics

The secondary screening of selective isolates was further characterized for different plant growth-promoting activities like Nitrogen fixation(N_2), phosphate (P) and potassium (K) solubilization,siderophore production and indole acetic acid (IAA) production.

Nitrogen fixation

Nitrogen fixation by bacterial isolates was screened out using Jensen's medium. Each activated bacterial culture was spotted (1 µl) on Jensen's agar medium and incubated at 28±2°C for 4 days. The plate was monitored, and the growths of nitrogen-fixation bacteria were recorded daily.

Phosphate solubilization

Phosphate solubilization by bacterial isolates was screened by using Pikovskaya's agar supplemented with bromophenol blue. Phosphate as tricalcium phosphate is present in medium. Each bacterial isolate with 1 µl of inoculum size was spotted on Pikovskaya's agar and incubated at 28±2°C for 8 days. The plate was monitored and observed for the formation of a zone around the colony due to the solubilization of tricalcium phosphate by bacteria.The solubilizing index of the bacteria was calculated.

$$\text{Solubilizing index (S.I.)} = \frac{z+c}{c}$$

Where Z = solubilization zone (mm) and C = colony diameter (mm).

Potassium solubilization

Potassium solubilization by bacterial isolates was screened by using Aleksandrow agar. Potassium, as potassium alumino silicate, is present in the medium. Each bacterial culture was spotted on Aleksandrow agar and incubated at 28±2°C for 8 days. The plate was monitored and observed for zone development around the colony due to the solubilization of potassium alumino silicate. The solubilization index of bacteria was measured as mentioned above.

Siderophore production

Bacterial isolates were screened out for siderophore production by using King's B medium supplemented with CAS dye. The 1 µl bacterial cultures were spotted on King's B medium and incubated at 28±2°C for 6 days, respectively. The plate was monitored for zone development around the colony due to the uptake of iron by bacteria. The iron uptake index of bacteria was measured as mentioned above.

IAA production

Each bacterial strain was grown in 50 ml sugar tube containing 20 ml LB (Luria bertani) broth with 5 mM L-tryptophan and without L-tryptophan medium. A total 10 sugar tubes were incubated under shaking at 28°C, 120 rpm for 5 days. Then, the bacterial cultures were harvested by centrifugation at 10,100 rpm for 10 minutes. Bacterial supernatant was used to determine the concentration of IAA by using 2 ml Salkowski's reagent (7.5 ml 0.5 M $FeCl_3.6H_2O$, 150 ml conc. H_2SO_4, 250 ml distilled water) and incubated for 35 minutes in dark condition at room temperature. After incubation, pink-red colour was developed, indicating IAA production which was measured at 530nm using UV- visible spectrophotometer. The standard curve of IAA was prepared using known concentrations of IAA solution (10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg.ml^{-1}).

Antimicrobial susceptibility testing and Hemolytic activity

Susceptibility to 8 antimicrobial agents was performed using the disk-diffusion method in accordance with the Clinical and Laboratory Standards Institute recommendations (CLSI, 2013). Antimicrobial agents tested were (charge in µg/disk) Amikacin (30), Carbenicillin (100), Chloramphenicol (30), Ciprofloxacin (5), Cetotaxime (30), Gentamicin (10), Norfloxacin (10), Tobramycin (10).Hemolytic activity was determined by spotting the isolates on Brain Heart infusion (BHI) agar supplemented with 5% (v/v) blood and incubated for 48 h at 28°C, clearing zone around the colonies is indicative of the presence of hemolytic activity.

Seeds treatment

Vigna radiata L. seeds were collected from the local market for pot experiments. After thoroughly cleaning the seeds with tap water to remove all the debris and dust,seeds were further washed with sterile distilled water. The seeds were treated with 0.1% NaOCl solution for 3 minutes, followed by 70% ethanol for 3 times and rinsed with sterile distilled water 5 times.For bacterial inoculation, the seeds were incubated for 12h in the appropriate suspension of bacterial cultures and air dried aseptically in the laminar airflow and for control treatment, seeds were treated with water instead of bacterial suspensions.

Pot trial

The pot trial was conducted in pot bags with a height of 30.0 cm and a diameter of 14.0 cm between January and March 2023at the Department of Biosciences, Saurashtra University, under green-house.The average temperature of green-house was 35±2°C. For the pot preparation, agricultural soil was taken from a nearby village. Each pot was filled with approximately 12 Kg of non-sterile soil. *Vigna radiata* L. seeds treated with individual bacterial cultureswere transplanted at a depth of 2 cm. Pots were prepared and named as control, B-1, B-6, B-10 and B-11, respectively. The plants from each pot were harvested after 30 days of pot inoculation to studythe morphological characteristics of plants (fresh weight, total length, shoot length, root length, root branch number,dry weight, and leaf number).

RESULTS AND DISCUSSION

Isolation and primary screening of bacteria

Total of 47 bacteria were isolated from an agriculture soil sample, of which 39, 9, 6 and 9 isolates were positive for N_2-fixation, phosphate solubilization, potassium solubilization and siderophore production, respectively.

Secondary screening of plant growth-promoting bacteria

Four bacterial isolates (B-1, B-6, B-10 and B-11) were selected for further study because of their multiple plant growth-promoting characteristics like N_2-fixation, phosphate solubilization, potassium solubilization and siderophore production (Fig.1).Two isolates B-1 and B-6 were Gram-positive, while B-10 and B-11 were Gram-negative bacteria.

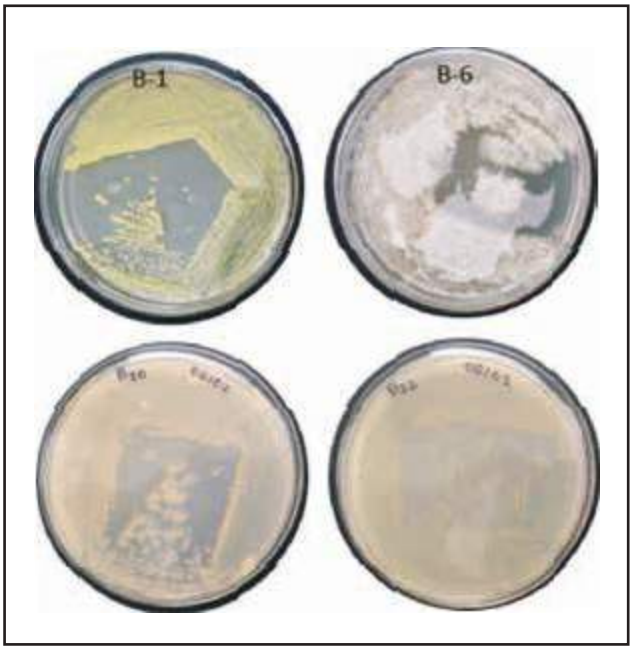

Figure 1: Pure isolates (B-1, B-6, B-10 and B-11) of plant growth promoting bacteria.

Nitrogen fixation

All 4 isolates were able to grow on nitrogen-free Jensen's medium, indicating that all bacteria could fix atmospheric $N_2$for their growth requirement (Fig. 2 A).Ahemad &Kibret, 2014 suggested that nitrogen is the most important and limiting nutrient for plant growth as it is the fourth most primary element present in plant's dry mass. Nitrogen is an essential component of enzymes and structural proteins in living organisms (Marschner, 1995). *Pseudomonas aeruginosa*also showed nitrogen fixation on Jensen media (Roychowdhury et. al., 2019). Biological nitrogen fixation (BNF) is a major source of nitrogen for plants as part of environmentally friendly agricultural practices.

Phosphate solubilization

Total 3 isolates (B-1, B-10, B-11) were positive for phosphate solubilization (Fig. 2 B).The maximum phosphate solubilization index was observed by B-11 (8.42), followed by B-10 (6.0) and B-1 (5.0).Bacteria which are able to produce organic acid were able to convert insoluble form of soil phosphate into soluble form, making it available for plant uptake and nutrition.*Pseudomonas sp.* MS16 and *Enterobacter sp.* MS32 isolated from rhizospheric soil shows 3.2 to 5.8 solubilizing index(Suleman et. al., 2018). B-11 shows a high solubilizing index compared to the above isolates. P is abundant in soils in both organic and inorganic forms, and its availability is restricted to plants as it occurs mostly in insoluble forms (Pradhan and Sukla, 2005). Thus, releasing these fixed and insoluble forms as soluble forms is a very important factor in increasing soil P availability.

Potassium solubilization

Three bacterial isolates (B-1, B-10, B-11) were positive for potassium solubilization (Fig. 2 C).The maximum potassium solubilization index was observed by B-11

(27.66), followed by B-1(7.5) and B-10 (4.33).Potassium (K) solubilization is done by different microorganisms such as saprophytic bacteria, fungal strains and actinomycetes (Ahmad et al., 2016; Bakhshandeh et al., 2017; Gundala et al., 2013; Meena et al., 2014). There is strong evidence that soil bacteria can transform soil K into the forms that are available to plants effectively (Meena et al., 2015a; Meena et al., 2014).

Siderophore production

Two bacterial isolates (B-1 and B-10) were positive for siderophore production (Fig. 2 D). The maximum iron uptake index was observed by B-1 (6), followed by B-10 (4.5).SJ-3 isolated from soybean rhizosphere shows 4.2 iron uptake index (Jain et. al., 2016). Plants need Fe^{2+} ions for various internal metabolic reactions, including photosynthesis and atmospheric nitrogen fixation. Even though iron is one of the most prevalent elements in the crust of the Earth, plant roots have very little opportunity to absorb it because iron ions in the environment quickly oxidize from Fe^{2+} to Fe^{3+}, rendering them intractable.

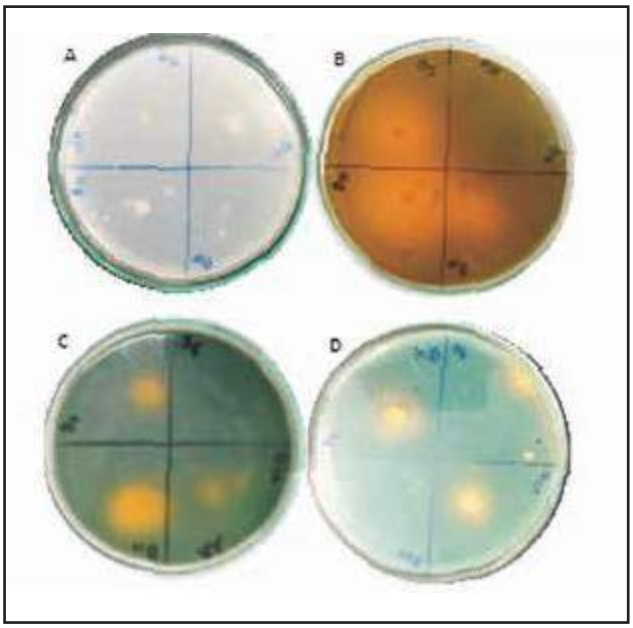

Figure 2: Plant growth-promoting activity of four bacterial isolates: (A) Nitrogen fixation, (B) Phosphate solubilization, (C) Potassium solubilization and (D) Siderophore production.

Indole acetic acid

All bacterial isolates were able to produce IAA in the occurrence of L-tryptophan as well as in the nonappearance of L- tryptophan; however, in the nonappearance of L-tryptophan, the amount of IAA production is less compared to in the occurrence of L-tryptophan (Fig. 3). In the occurrence of L-tryptophan, highest amount of IAA production was observed by B-11 with 22 µg/ml at 120 h while in the absent of L- tryptophan, B-10 show maximum IAA production with 4.5 µg/ml at 96 h. IAA is also stimulated by microbial biosynthesis in soil, which is enhanced by the production of tryptophan from the root exudate or decaying cells (*Benizri* et al. 1998).

The most bacteria that interact with plants are able to produce IAA, which is likely as a result of the fact that IAA synthesis gives those bacteria a selection advantage in the environment (Kim et al. 2011). Furthermore, the high proportion of PGPB that possess the ability to biosynthesize IAA may indicate that bacterial IAA functions in ways that go beyond how IAA interacts with plants (Patten et al., 2013).

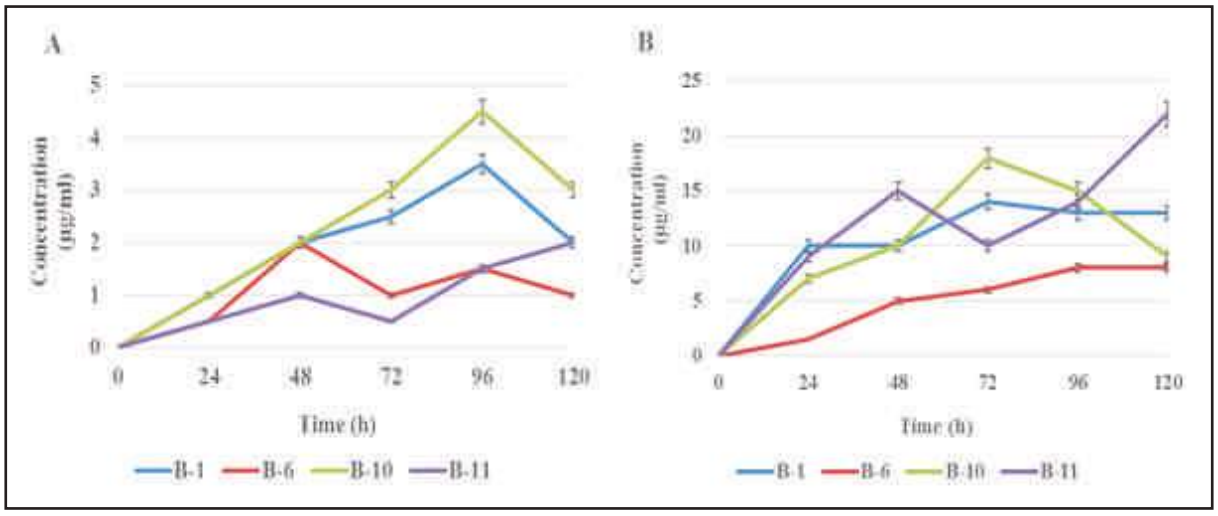

Figure 3: IAA production by PGPB (A) IAA production in the nonappearance of L-tryptophan; (B) IAA production in the occurrence of L- tryptophan.

Antimicrobial susceptibility testing and Hemolytic activity

All the isolates were sensitive against antibiotics except B-6 which show resistance against Carbenicillin. Highest antibiotic sensitivity was observed by B-10 (34mm) on Ciprofloxacin, while lowest antibiotic sensitivity was observed by B-11 (10mm) against Carbenicillin and Cetotaxime (Table 1).All isolates shows negative result for hemolytic activity as no hemolytic zone was observed around colony (Fig. 4).

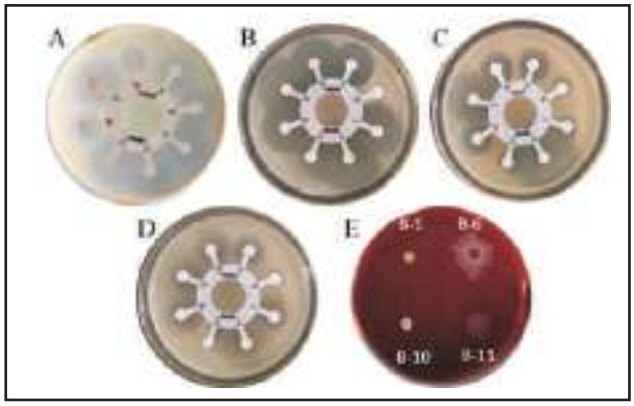

Figure 4: Antimicrobial susceptibility test for different isolates A) B-1, B) B-6, C) B-10, D) B-11 andE) Hemolytictest.

Table 1 : Antimicrobial susceptibility test against isolates.

Isolate	Zone of Inhibition (mm)							
	AK	CB	C	CIP	CTX	GEN	NX	TOB
B-1	22	18	21	22	21	17	26	13
B-6	23	0	24	32	13	24	31	25
B-10	18	12	13	34	11	18	25	19
B-11	18	10	12	23	10	15	19	18

Note: Amikacin (AK), Carbenicillin (CB), Chloramphenicol (C), Ciprofloxacin (CIP), Cetotaxime (CTX), Gentamicin (GEN), Norfloxacin (NX), Tobramycin (TOB).

Pot study

Isolates were examined in the primary test for their potential to promote plant growth. Further research was done on their biological influenceon the improvement of the mung bean plant. The effect of pure isolates of B-1, B-6, B-10, and B-11 was tested, and they showed a significant increase in plant growth compared to the control. Under the influence of various bacterial treatments, different plant responses were detected. Compared to the other isolates, B-11 culture showed the most promising results overall.

Plant growth promoting activity on *Vigna radiata* L.

In pot trial conditions, the effect of B-1, B-6, B-10, and B-11 bacterial treatments was studied on *Vigna radiata* L. and the influence of different isolates on the growth of mung plants over control was measured (Table 2). After the 30 days of pot trial, different plant parameters likeroot length, total length, root branch, leaf numbers and dry weight were improved in bacterial-treated plants. Compared to control plants, leaves were larger and longerin treated plants. Plantstreated with B-1showed significant increases in the fresh weight, total length, shoot length, root length, root branch number and dry weight by 57.14, 21.57, 14.29, 30.43, 333.33 and 11.43%,respectively, compared to the control plant. Likewise, plantstreated with B-10 showed significant improvement in the shoot length, total length, root length,root branch number,leaf number anddry weight by 14.29, 17.65, 21.74, 300, 37.50 and51.43%,respectively, over control.The plant treated with B-11 culture showed considerable growth in the fresh weight, shoot length, root length, total length, root branch number,dry weight, and leaf number by 76.43, 21.43, 39.13, 29.41, 400,71.43 and 37.50 % compared to thecontrol treatment after 30^{th} day (Fig.5).

One possible explanation for the variation in fresh and dry weights between the treated and control groups could be the PGPB's ability to give the treated plant a greater water-holding capacity.All plants utilize nitrogen (N) in the form of NO_3^- and NH_4^+. It is imperative for proper plant development, which significantly increases production and quality by playing a vital role in plant biochemical and physiological functions (Leghari et al., 2016). The amount of insoluble phosphorus in soil that is available for plant uptake is increased by phosphate solubilizing microbes (PSM). PSMs solubilize and mineralize insoluble organic phosphorus. Therefore, inoculation of PSM in soil or crops is a promising technique to boost plant phosphorus uptake and reduce the use of environmentally harmful chemical fertilizers (Alori et al., 2012).When compared to control plants, the application of PGPB enhanced vegetative growth parameters, most notably in the leaf area (Abdelaal et al., 2021). Our findings support many research that has documented the positive impact of the PGPB on promoting plant development.

Figure 5 : The top row illustrates the growth response of *Vigna radiata* L. plants treated with the plant growth-promoting isolate. The bottom row highlights root branch development and compares root length between the control and B-11treated plants.

Table 2 : Effect of PGPB on growth of *Vigna radiata* L.

Vegetative parameters	Treatments				
	Control	B-1	B-6	B-10	B-11
Fresh weight (g)	1.40	2.20	1.5	2.29	2.47
Total length (cm)	51	62	53	60	66
Root length (cm)	23	30	25	28	32
Shoot length (cm)	28	32	28	32	34
Root branch number (n)	3	13	10	12	15
Leaf number (n)	8	8	8	11	11
Dry weight (gm)	0.35	0.39	0.43	0.53	0.6

CONCLUSION

The present study demonstrates rhizospheric potential bacteria with plant growth-promoting activities like nitrogen fixation, phosphate and potassium solubilization, siderophore production and IAA production. Plant growth was greatly boosted when isolates B-1, B-6, B-10, and B-11 were applied in pure culture form instead of control treatment. *Vigna radiata* L. demonstrated significant improvements in terms of fresh weight, dry weight, shoot length, root length, number of root branches, and number of leaves. The majority of soils worldwide lack nutrients that are accessible to plants; therefore, adding bioinoculants would improve soil fertility by increasing the accessibility of nutrients. As a result, less chemical fertilizer will be used, and sustainable agriculture will be promoted. The plant growth-promoting bacterial isolates of rhizospheric soils are potential candidates for use as bioinoculants and biofertilizers.

Conflict of interest

The authors declared no conflict of interest.

REFERENCE

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King saud University-science*, 26(1), 1-20.

Ahmad, M., Nadeem, S. M., Naveed, M., & Zahir, Z. A. (2016). Potassium-solubilizing bacteria and their application in agriculture. Potassium solubilizing microorganisms for sustainable agriculture, 293-313.

Azziz, G., Bajsa, N., Haghjou, T., Taulé, C., Valverde, Á., Igual, J. M., & Arias, A. (2012). Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. *Applied Soil Ecology*, 61, 320-326.

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, cell & environment*, 32(6), 666-681.

Bakhshandeh, E., Pirdashti, H., &Lendeh, K. S. (2017). Phosphate and potassium-solubilizing bacteria effect on the growth of rice. *Ecological Engineering*, 103, 164-169.

Benizri, E., Courtade, A., Picard, C., & Guckert, A. (1998). Role of maize root exudates in the production of auxins by Pseudomonas fluorescens M. 3.1. *Soil Biology and Biochemistry*, 30(10-11), 1481-1484.

CLSI (2013) Clinical and Laboratory Standards Institute. Performance stan dards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-S22. Wayne, Pennsylvania

Dimkpa, C., Weinand, T., & Asch, F. (2009). Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant, cell & environment*, 32(12), 1682-1694.

Drobek, M., Frąc, M., & Cybulska, J. (2019). Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress-A review. *Agronomy*, 9(6), 335.

Efthimiadou, A., Katsenios, N., Chanioti, S., Giannoglou, M., Djordjevic, N., & Katsaros, G. (2020). Effect of foliar and soil application of plant growth promoting bacteria on growth, physiology, yield and seed quality of maize under Mediterranean conditions. *Scientific reports*, 10(1), 21060.

Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012.

Gray, E. J., & Smith, D. L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil biology and biochemistry*, 37(3), 395-412.

Grover, M., Ali, S. Z., Sandhya, V., Rasul, A., &Venkateswarlu, B. (2011). Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology and Biotechnology*, 27, 1231-1240.

Gundala, P. B., Chinthala, P., & Sreenivasulu, B. (2013). A new facultative alkaliphilic, potassium solubilizing, Bacillus Sp. SVUNM9 isolated from mica cores of Nellore District, Andhra Pradesh, India. Research and Reviews. *J Microbiol Biotechnol*, 2(1), 1-7.

Hii, Y. S., San, C. Y., Lau, S. W., & Danquah, M. K. (2020). Isolation and

characterization of phosphate solubilizing microorganisms from peat. *Biocatalysis and Agricultural Biotechnology*, 26, 101643.

Jain, S., Kumari, S., Vaishnav, A., Choudhary, D. K., & Sharma, K. P. (2016). Isolation and characterization of plant growth promoting bacteria from soybean rhizosphere and their effect on soybean plant growth promotion. *International Journal of Advanced Scientific and Technical Research*, *5*(6), 397-410.

Kim, Y. C., Leveau, J., McSpadden Gardener, B. B., Pierson, E. A., Pierson III, L. S., & Ryu, C. M. (2011). The multifactorial basis for plant health promotion by plant-associated bacteria. *Applied and Environmental Microbiology*, 77(5), 1548-1555.

Leghari, S. J., Wahocho, N. A., Laghari, G. M., HafeezLaghari, A., MustafaBhabhan, G., HussainTalpur, K., & Lashari, A. A. (2016). Role of nitrogen for plant growth and development: A review. *Advances in Environmental Biology*, *10*(9), 209-219.

Marschner, C. (1996). H. Marschner, Mineral Nutrition of Higher Plants, Academic Press, London (1995), p. 889,(ISBN 0-12-473543-6). Price: 29.95 Pound Sterling.

Marschner, H. (1995). Nutrition of higher plants. *Nutrition of Higher Plants*, 4.

Meena, V. S., Maurya, B. R., & Bahadur, I. (2015). Potassium solubilization by bacterial strain in waste mica. *Bangladesh Journal of Botany*, 43(2), 235-237.

Meena, V. S., Maurya, B. R., & Verma, J. P. (2014). Does a rhizospheric microorganism enhance K^+ availability in agricultural soils?. *Microbiological research*, 169(5-6), 337-347.

Mushtaq, Z., Faizan, S., & Hussain, A. (2021). Role of microorganisms as biofertilizers. *Microbiota and Biofertilizers*: A Sustainable Continuum for Plant and Soil Health, 83-98.

Naveed, M., Khalid, M., Jones, D. L., Ahmad, R., & Zahir, Z. A. (2008). Relative efficacy of Pseudomonas spp., containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of organic fertilizer. *Pak. J. Bot*, 40(3), 1243-1251.

Patten, C. L., Blakney, A. J., & Coulson, T. J. (2013). Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. *Critical reviews in microbiology*, 39(4), 395-415.

Pradhan, N., &Sukla, L. B. (2006). Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology*, 5(10).

Roychowdhury, R., Qaiser, T. F., Mukherjee, P., & Roy, M. (2019). Isolation and characterization of a Pseudomonas aeruginosa strain PGP for plant growth promotion. *Proceedings of the national academy of sciences, India section b: biological sciences*, *89*, 353-360.

Suleman, M., Yasmin, S., Rasul, M., Yahya, M., Atta, B. M., & Mirza, M. S. (2018). Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. *PloS one*, 13(9), e0204408.

Verma, P., Yadav, A. N., Kumar, V., Singh, D. P., & Saxena, A. K. (2017). Beneficial plant-microbes interactions: biodiversity of microbes from diverse extreme environments and its impact for crop improvement. *Plant-microbe interactions in agro-ecological perspectives*: volume 2: microbial interactions and agro-ecological impacts, 543-580.