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In vitro detection of plant growth-promoting traits in bacteria isolated from the rhizosphere of *Vigna unguiculata* (L.) walp

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Abstract
Plant growth is a major index in sustainable crop production and food security. Plant growth is controlled by a number of factors that often create stress conditions. Among the prominent biotic factors are microorganisms including plant growth-promoting rhizobacteria. In the present study, the rhizosphere of cowpea was explored with a view to detecting and identifying plant growth-promoting bacteria. Soil samples were collected from the cowpea root zone and subjected to microbial analysis using standard procedures. The isolates were screened for their ability to produce and exhibit plant growth properties. A total of 17 bacterial species were isolated and identified as *Paenibacillus* spp. (11.76%), Pseudomonas spp. (11.76%), *Corynebacterium* spp. (11.76%), Micrococcus spp. (11.76%), *Paraburkholderia sprentiae* CRA1 (5.88%), *Burkholderia gladioli* CRA4 (5.88%), *Arthrobacter* sp. URR2 (5.88%) with the predominance of Bacillus spp. (35.29%). Some species including Pseudomonas aeruginosa CRD2, *Micrococcus varians* CRA3 and *Corynebacterium kutsceri* CRB5 were able to show multiple traits including cellulase and pectinase production; ammonium and indole acetic acid (IAA) production and phosphate solubilization. *Paraburkholderia sprentiae* CRA1 showed the highest (144.4%) phosphate solubilization efficiency followed by *Paenibacillus validus* CRD1 (137.5%) and *Paenibacillus alvei* CRB4 (133.3%). As majority of the isolates were cellulase, pectinase, and ammonium producers, more than 50% of the isolates were phosphate solubilizers. The ability of the bacteria to express one or more of these traits is an indication of their ability to enhance plant growth and performance. The need to explore their individual and additive effect with a view to harnessing their potential is therefore recommended.

Keywords: bacteria, rhizosphere, phosphate solubilization, plant

Introduction

Plants like animals, live amidst a complex microbial community which determines the overall plant health and performance [1]. Plants and microorganisms have holobiant connections that are maintained through bio-mineralization and synergistic co-evolution, resulting in a high potential for enhancing soil quality and fertility [2, 3]. Plant microbiota is present in their endosphere, rhizosphere and phyllosphere which consist of the core microbiome, major microbiome and the endosphere microbiomes [4]. Plant biota are not absolutely beneficial, but some species are harmful and disastrous. There are a number of evidences that demonstrated the parasitic, pathogenic and even predatory interaction between plants and their microbiota [5, 6]. Within the rhizosphere, the intimacy between plants and microbes is high and extends within a gradient as it moves away from the roots. As such, there is a varying microbial abundance and diversity in which substantial microbial alteration in the soil is pronounced adjacent to roots and subside as it far away [4]. The dynamics in the rhizosphere greatly affect plant life and previous studies have indicated that microorganisms like bacteria and fungi play a vital role in promoting plant health through enhanced protection against environmental stresses [7].

Utilizing microorganisms that promote plant growth is one method for growing plants in arid environments with a view to palliating biotic and abiotic stress. A group of bacteria known as plant growth-promoting bacteria (PGPB) are present in the rhizosphere near the root systems of plants, both at the root surface and in endophytic associations. These bacteria can either directly or indirectly promote plant growth under favorable, biotic, or abiotic stress circumstances [8]. The generation of phytohormones such auxins, cytokinins, and gibberellins, the solubilization of mineral phosphates, and iron sequestration by bacterial siderophores are all known methods exploited by PGPB. Numerous PGPB have been demonstrated to mitigate the impacts of drought stress in plants by lowering plant ethylene levels, which are often elevated by unfavorable circumstances [9]. Plant growth-promoting bacteria application is regarded as a viable, synergistic biological strategy to address agricultural production's water shortage. PGPB easily colonize the rhizosphere around plant roots and form close relationships with hosts. Through a number of mechanisms, these interactions frequently result in an improvement in crop productivity and the mitigation of biotic and abiotic pressures [10]. But for a successful application, especially in soils under drought stress, the ability of inoculated bacteria to survive, outcompete the native microflora, and colonize the rhizosphere remains crucial. This is because microorganisms that are not adapted to high water tension will perish under these unfavorable conditions. Therefore, the drought-tolerant bacteria may have an edge over others in thriving in new arid environments in large enough numbers to have positive impacts on plants [11].

Plant stress is one of the major agricultural problems reducing crop yield in arid and semiarid regions of the world. Changes in mean global air temperature and precipitation patterns due to climate change are creating more stress to plants including longer drought periods [12]. At present, strategies to increase the ability of plants to

tolerate stress involve the use of water-saving irrigation, traditional breeding, and genetic engineering of stress-tolerant transgenic plants. Unfortunately, these methods are highly technical and labor-intensive, and thus difficult to apply in practice. In order to address this global challenge, research has focused on improving germplasm and developing crop management practices to increase water use efficiency and nutrient acquisition [13]. However, recent attention has turned to the application of beneficial microorganisms that mediate stress tolerance and improve plant water-use efficiency [14]. Recently, soil quality rejuvenation and plants growth enhancement by PGPR had been an area actively exploited for improved agriculture productivity in many parts of the world. It is against this backdrop, the present study explored the rhizosphere of cowpea – an important tropical food crop with a view to prospecting PGPB for improved plant activity.

Materials and methods

Study site

The experiments were conducted at the Botanical Garden of the Isa Kaita Collage of Education, Dutsin-ma (coordinates: Lat. 12° 99' N; Long. 7° 63' E). Laboratory experiments were carried out at Biology Laboratory, Department of Biology in the same Institution. Sample collection Seeds of Vigna unguiculata (cowpea) used were procured from Pastoral Research Institute Ahmadu Bello University Zaria.

Sample collection

Soil samples were aseptically collected from the plant's rhizosphere as described by Ismail et al. [15]. The samples were placed in sterile sample containers and transported to the laboratory immediately for analyses. Samples that could not be analyzed within 1 hour after collection were preserved at low temperature (± 2℃).

Isolation and identification of bacterial species

Rhizosphere bacteria were isolated by inoculating serially diluted (×108 cfu/g) soil samples in nutrient agar (Oxoid) after 24 hours incubation. Distinct bacterial colonies were selected and sub-cultured to obtain pure cultures. The pure isolates were preserved for identification. The isolates were characterized using cultural, morphological and biochemical characteristics as described by Benson et al. [16] based on the schemes of Barrow and Feltham [17]. The bacterial characteristics were compared with the database available on https://www.tgw1916.net/bacteria_logare_desktop.html for identification.

Determination of plant growth promotion traits

Cellulase activity

The identified bacterial isolates were screened for cellulase enzyme production using carboxy methyl cellulose (CMC) agar as described by Zaghloul et al. [18]. On CMC agar, aliquots of the bacterial suspensions were plated. The plates were incubated for five days at 30 °C.

After the incubation period, a 15-minute flood of an aqueous Congo red solution (1% w/v) was applied to the culture surface. After pouring out the Congo red solution, 15 minutes of flooding with 1M NaCl was used to further treat the plates. Degradation of the cellulose was demonstrated by the conspicuous zone of hydrolysis that appeared.

Pectinase activity

Petri plates were supplied with prepared pectin agar medium. With the aid of a cork-borer, halls were aseptically created on the agar after solidification. The tested isolates were added to the halls in 0.5 ml volumes, and they were cultured for 4 days at 30°C. To find the clearance zone, iodine-potassium/iodide solution was added to the plate's surface. The diameter of the clear zone around the halls, measured in millimeters, was used to determine the enzyme activity [19].

Phosphate solubilization

Phosphate-solubilizing ability of the bacteria isolates was determined on Pikovskaya agar medium. The isolates were spotted onto Pikovskaya agar and incubated at 30°C for 3 days. The presence of halo zone around the bacterial colony was considered as indicator for positive ability for inorganic phosphate solubilization. The results were expressed as solubilization efficiency (SE) as described by Zaghloul et al. [18].

SE = (Solubilization diameter)/(Growth diameter) × 100

Indole Acetic Acid (IAA) production

The ability of the isolates to produce IAA was investigated using the methods of Shrivastava et al. [20]. Prepared and poured into sterile petri dishes was a minimal salt agar plate with 0.1g/mL of tryptophan. A 2 cm diameter hole was made using a sterile cork borer after proper solidification. Each hole received an overnight culture (0.2ml) of the isolates, which was then placed and kept at 30oC in an incubator. The cultures were carefully removed from the holes after an overnight growth period with tissue paper, and 0.2 ml of Salkowsky reagent (12 g FeCl per liter in 7.9 M H2SO4) was then added.

Following reagent addition, a pink halo zone surrounded the holes, which was measured, and the diameter of the hole as well as the combined halo zone diameter of all strains were then determined. The IAA production index of each strain was calculated by the formula:

$$\text{IAA} = \frac{\text{Total halo zone diameter} - \text{Hole diameter}}{\text{Hole diameter}}$$

HCN production

Production of HCN was assessed as described by Zaghloul et al. [18]. The endophytic isolates were grown in nutrient agar supplemented with glycine (4.4 g L-1). One sheet of a sterilized Whatman filter paper was immersed in 1% picric acid in 10% sodium carbonate for 1 min then placed on the surface of the plate. The plates were sealed with parafilm and incubated at 28 ± 2°C for 2 days. Development of reddish-brown color on the Whatman filter paper indicated production of HCN.

Production of ammonia

The bacterial isolates were tested for the ability to produce ammonia in nutrient broth.

Freshly grown bacterial cultures were inoculated in 10ml nutrient broth and incubated at 30°C for 48 hours in a rotary shaker at 200 rpm. After the incubation period, 0.5 ml of Nessler's reagent was added and thoroughly mixed in each tube. The development of yellow-brown color indicated positive reaction for ammonia [21].

Results

In this study, a total of 17 bacterial species were isolated from the rhizosphere of Vigna unguiculata. Based on their morphological and biochemical properties, they were identified to consist of predominantly Bacillus spp. (35.29%), Paenibacillus spp. (11.76%), Pseudomonas spp. (11.76%), Corynebacterium spp. (11.76%) and Micrococcus spp. (11.76%). Other species identified include Paraburkholderia sprentiae CRA1, Burkholderia gladioli CRA4 and Arthrobacter sp. URR2 with 5.88% occurrence rate each as shown in Figure 1.

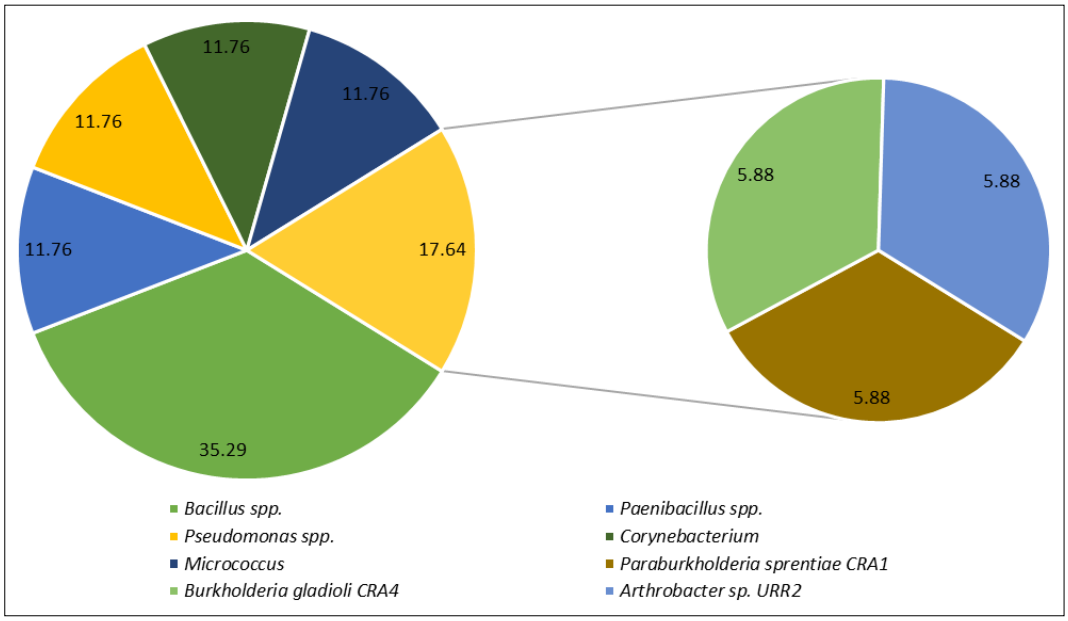

Fig 1: Occurrence rate of bacteria isolated from the rhizosphere of Vigna unguiculata. The Bacillus isolates include Bacillus licheniformis CRC1, Bacillus subtilis CRC2, Bacillus cereus URR1, Bacillus cereus CRB1, Bacillus atrophaeus CRB2 and Bacillus niacini CRA2.

The ability of the bacterial species to promote plant growth was investigated (Table 1). Many of the rhizosphere and non-rhizosphere bacteria were able to exhibit one or more PGP trait. Pseudomonas aeruginosa CRD2, Micrococcus varians CRA3 and Corynebacterium kutsceri CRB5 were able to show multiple PGP traits including cellulase and pectinase activity, ammonium and indole acetic acid (IAA) production and phosphate solubilization. Paenibacillus azotofixans CND4 showed highest (142.9%) phosphate solubilization efficiency followed by Enterococcus sp. CNC3, Pseudomonas aeruginosa CRD2, and Bacillus niacini CRA2, and Micrococcus varians CRA3 and Burkholderia gladioli CRA4 with 128.6% solubilization efficiency each. Production of cellulase, pectinase, ammonium, IAA and phosphate solubilization was observed in 9, 9, 9, 4 and 10 bacterial species respectively out of the 17 isolates. Pseudomonas aeruginosa CRD2 could be described as the best isolate with PGP traits considering the fact that it had the highest IAA index (4.0) in addition to possessing all the investigated traits. On the other hand, majority of the isolates lacking PGP traits are members of the genus Bacillus.

Table 1: Plant growth promoting traits of rhizosphere bacteria

Isolate	Plant Growth promotion trait		
	Cellulase activity	Pectinase activity	Ammonium Production
Bacillus licheniformis CRC1	-	+	+
Bacillus subtilis CRC2	-	-	-
Bacillus cereus URR1	-	-	-
Paenibacillus validus CRD1	+	-	+
Pseudomonas aeruginosa CRD2	+	+	-
Pseudomonas aeruginosa CRD3	-	-	+
Micrococcus varians CRD4	+	-	-
Bacillus cereus CRB1	-	-	-
Bacillus atrophaeus CRB2	+	-	-
Corynebacterium xerosis CRB3	-	-	
Paenibacillus alvei CRB4	+	+	+
Corynebacterium kutsceri CRB5	+	+	+
Paraburkholderia sprentiae CRA1	+	+	+
Bacillus niacini CRA2	-	+	-
Micrococcus varians CRA3	+	+	+
Burkholderia gladioli CRA4	+	+	+
Arthrobacter sp. URR2	-	+	+

Table 2: Plant growth promoting traits of rhizosphere bacteria

Isolate	Plant Growth promotion traits			
	Phosphate		Indole Acetic Acid (IAA)	
	Solubilization	SE (%)	Production	IPI
Bacillus licheniformis CRC1	+	109.1	-	-
Bacillus subtilis CRC2	-	-	-	-
Bacillus cereus URR1	-	-	-	-
Paenibacillus validus CRD1	+	137.5	-	-
Pseudomonas aeruginosa CRD2	+	128.6	+	4.0
Pseudomonas aeruginosa CRD3	+	118.75	-	-
Micrococcus varians CRD4	-	-	-	-
Bacillus cereus CRB1	-	-	-	-
Bacillus atrophaeus CRB2	-	-	-	-
Corynebacterium xerosis CRB3	-	-	-	-
Paenibacillus alvei CRB4	+	133.3	-	-
Corynebacterium kutsceri CRB5	-	-	+	2.6
Paraburkholderia sprentiae CRA1	+	144.4	-	-
Bacillus niacini CRA2	+	128.6	-	-
Micrococcus varians CRA3	+	128.6	+	2.0
Burkholderia gladioli CRA4	+	128.6	-	-
Arthrobacter sp. URR2	+	116.7	+	2.2

SE: Solubilization efficiency; IPI: Indole Acetic Acid production Index

Discussion

Rhizospheric bacterial species isolated and identified in this study indicated that Bacillus species were the most abundant. Bacillus spp. are among the prominent bacteria that inhabit soil due to their ability to withstand the uncertain soil dynamics. Ability to form endospores have been described as one of their major response to soil adverse conditions. When the conditions become favorable, the population surge immediately and provide the species an advantage to predominate. Previous and recent studies have indicated that Bacillus species formed the dominant populations in the rhizospheres of plants [22, 23]. The occurrence of Paenibacillus spp. demonstrated its possible symbiotic relationship and could be a good candidate for PGP. It has been established that Paenibacillus is a well-known plant growth-promoting bacteria that contributes to cowpea yield [24]. Recent studies by dos Santos et al. [25] have reported the presence of Paenibacillus in the rhizosphere of lima bean with ability to promote plant growth through antibiotic secreation and nitrogen fixation. Similarly, the occurrence of Pseudomonas spp., Corynebacterium spp. and Micrococcus spp. in different plant rhizospheres have been documented [26]. Study by Ghodsalavi et al. [27] indicated that among many bacterial species in the rhizosphere of Valeriana officinalis, the population of Pseudomonas spp. dominated the rhizosphere and showed a high capability to be used as a biofertilizer. The presence of Paraburkholderia sprentiae [28], Burkholderia gladioli [29] and Arthrobacter sp. [30] as rhizosphere bacteria have all been reported.

Bacterial species identified in this study were observed to possess one or more plant growth promotion traits. Traits including cellulase, pectinase, ammonium and indole acetic acid (IAA) production and phosphate solubilization were expressed by Pseudomonas aeruginosa CRD2, Micrococcus varians CRA3 and Corynebacterium kutsceri CRB5 among the rhizosphere bacteria. Ability of microbial species to express any of the aforementioned properties is an indication of its capability to promote plant growth and performance. Ajmal et al. [8] showed that diverse groups of bacteria thrive in environments which may have PGP ability even under myriad stress conditions. Out of the 17 bacterial isolates, 52.94% (9) and 52.94% (9) were able to produce cellulase and pectinase respectively.

Recent studies by Bhattacharyya et al. [31] reported that 26.6% of 30 rhizospheric isolates were cellulase producers. Bhadrecha et al. [32] have also reported 17 rhizobacteria capable of cellulase and pectinase production including Bacillus, Paenibacillus, Brevibacillus and Pseudomonas from the rhizosphere of Hippophae rhamnoides and thus, support the present findings. As cellulases help in biocontrol of fungal phyto-pathogens by promoting fungal cell wall degradation, pectinases play an important role in root invasion by bacteria in the rhizosphere [33]. In addition, cellulase producing bacteria improve nutrient recycling in the rhizosphere by degrading organic carbon residues which improve soil health and plant growth [34].

Similarly, 52.94% of the rhizospheric bacteria were observed to produce ammonia. Most of the species in the rhizosphere were Bacillus, Paenibacillus, Micrococcus, Arthrobacter, Pseudomonas and Burkholderia species. Bacillus and Pseudomonas spp.

are believed to be among potent ammonia producers in the soil. Previous studies by Yadav et al. [35] have reported that 100% of Bacillus and Pseudomonas spp. among their isolates were ammonia producing. Singh et al. [36] have isolated ammonia producing bacteria belonging to the genera Enterococcus, Arthrococcus and Bacillus species. Fouda et al. [37] have also reported the ability of Bacillus subtilis, Paenibacillus barengoltzii, and Burkholderia cepacia isolated from the leaves of Pulicaria incisa to produce ammonia. Alkahtani et al. [38] have shown that most of the bacteria isolated from Fagonia mollis and Achillea fragrantissima were ammonia producing and capable of promoting the growth of Zea mays in greenhouse trials. Olayemi and Odedara [39] have also reported Staphylococcus spp. isolated from Nigerian rice varieties as potent ammonia producers. It has been established that bacteria hydrolyze urea to carbon dioxide and ammonia which is used by plants

as source of nitrogen [38, 40]. The bacteria that produce beneficial metabolites for plant, including ammonia, promote plant growth by inducing roots and shoots elongation and overall plant weight [33]. Indirectly, PGPB lower the ethylene levels of its host plant by converting its immediate precursor (1-aminocyclopropane-1-carboxylate [ACC]) to α-ketobutyrate and ammonia, there by promoting root growth [30] and alleviating plant abiotic stress [41].

Production of IAA which is a phytohormone is associated with increased plant growth and development. Although only 4 (23.53%) out of 17 isolates were able to produce IAA, this did not preclude the possibility of other isolates to be able to produce the phytohormone also; considering the fact that in this study, tryptophan was used as an inducer. Studies by Tang et al. [42] have shown that IAA can be produced by bacteria even in the absence of tryptophan because of the redundancy of the four IAA biosynthetic pathways in some microorganisms. Diverse group of bacterial species have been reported by Khatoon et al. [34] to produce IAA including some species reported in this study, which therefore supports our findings. Pseudomonas aeruginosa CRD2 and Corynebacterium kutsceri CRB5 have the highest IAA production index (4.0 and 2.6 respectively). Organisms capable of IAA production promote plant growth by stimulating increase in cell elongation in the short term and cell division and differentiation in the long-term [33].

Results in Table 2 showed that 58.82% of rhizospheric bacteria were able to solubilize inorganic phosphate. Some of the species with highest phosphate solubilization efficiency (SE) include Paraburkholderia sprentiae CRA1 (144.4) and Paenibacillus validus CRD1 (137.5). Previous studies have identified Burkholderia [43] and Paenibacillus [33] as some of the important phosphate solubilizing bacteria. Ability to produce organic acids like gluconic, isoveleric, lactic and acetic acids by PGPB have been linked to phosphate solubilization [33]. Emami et al. [43] suggested that phosphate solubilization by bacteria occurs in the rhizosphere and may play a great role in meeting-up plants' phosphate requirements.

Among all the isolates only Micrococcus varians CRA3 was able to express all the PGP traits examined in this study. This might be due to the fact that the isolates possess genetic backup responsible for expression of enzymes needed in the various metabolic processes.

3A number of studies have highlighted the fact that, ability to express PGP traits is both inherent and plasmid mediated [44]. Studies by Dastager et al. [45] have reported the ability of Micrococcus sp. with multiple PGP properties isolated from Indian forest in promoting the growth of cowpea. Their work is in conformity with the present findings.

Conclusion

Soil remains the largest reservoir of microorganisms and plant rhizosphere provide a favorable niche for microbial proliferation. In this study, the findings indicated the presence of bacterial species in the cowpea rhizosphere. The species had varying plant growth promotion abilities ranging from enzyme production, hormone secretion and nutrient mineralization. While some species are endowed with a single trait, many others possess multiple traits which may substantiate their presence as plant growth promoters. With the increase cost of crop production due to numerous biotic and abiotic challenges, harnessing the potentials of these bacteria will go a long way to supporting sustainable agriculture especially in developing countries.

References

1. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S and Smith DL. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. Front. Plant Sci,2018:9:1473. doi: 10.3389/fpls.2018.01473.
2. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D. Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol. 14:e1002352 2016. doi: 10.1371/journal.pbio.1002352.
3. Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological Research,2018:206:131-140. doi:10.1016/j.micres.2017.08.016.
4. Ismail HY, Farouq AA, Rabah AB, Muhammad AB, Allamin IA, Ibrahim UB *et al*. Microbe-Assisted Phytoremediation of Petroleum Hydrocarbons. In: J. A. Malik (Ed.) Handbook of Research on Microbial Remediation and Microbial Biotechnology for Sustainable Soil. IGI Global, USA, 2021.
5. Paungfoo-Lonhienne C, Rentsch D, Robatzek S, Webb RI, Sagulenko E, Nasholm T, Schmidt S *et al*. Turning the table: Plants consume microbes as a source of nutrients. PLoS One,2010:5(7):e11915. doi:10.1371/journal.pone.0011915 PMID:20689833.
6. Singh PP, Kujur A, Yadav A, Kumar A, Singh SK, Prakash B. Mechanisms of Plant-Microbe Interactions and its Significance for Sustainable Agriculture. In: PGPR Amelioration in Sustainable Agriculture, 2019. DOI: https://doi.org/10.1016/B978-0-12-815879-1.00002-1.
7. Saini R Sharma S. Climate resilient microbes in sustainable crop production. In: Contaminants in Agriculture and Environment: Health Risks and Remediation,2019. DOI: 10.26832/AESA-2019-CAE-0165-020.
8. Ajmal AW, Saroosh S, Mulk S, Hassan MN, Yasmin H, Jabeen Z, Nosheen A *et al*. Bacteria Isolated from Wastewater Irrigated Agricultural Soils Adapt to Heavy Metal Toxicity While Maintaining Their Plant Growth Promoting Traits. Sustainability,2021:13:7792. https://doi.org/10.3390/su13147792.
9. Arshad M, Shaharoona B, Mahmood T. Inoculation with Pseudomonas spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (Pisum sativum L.). Pedosphere,2008:18:611-620. doi: 10.1016/S1002-0160(08)60055-7.
10. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology,2006:57(1):233-266. doi:10.1146/annurev.arplant.57.032905.105159 PMID:16669762

11. Wu QS, Zou YN, Xia RX. Five Glomus species affect water relations of citrus tangerine during drought stress. Botanical studies,2007:48(2):147-154.
12. Paustian K, Lehmann J, Ogle S, Reay D, Robertson PG, Smith P. Climate smart soils. Nature,2016:532:49-57.
13. Ngumbi E, Kloepper J. Bacterial-mediated drought tolerance: current and future prospects. Appl. Soil Ecol,2016:105:109-125.
14. Vurukonda SS, Vardharajula S, Shrivastava M, Sk ZA. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol. Res.2016:184:13-24. doi: 10.1016/j.micres.2015.12.003.
15. Ismail HY, Ijah UJJ, Riskuwa ML, Allamin IA, Isah MA. Assessment of Phytoremediation Potentials of Legumes in Spent Engine Oil Contaminated Soil. European Journal of Environmental and Safety Sciences,2014:2(2):59-64.http://www.sci-nstitute.com/2014-Vol-2_-Issue-2.html?gen=214
16. Benson H. Microbiological applications laboratory manual. 8th ed. New York: McGraw-Hill Companies, 2002.
17. Barrow GI, Feltham KA. Cowan and Steel's Manual for Identification of Medical Bacteria. 3rd edition. Cambridge University Press, London, 1993.
18. Zaghloul RA, Abou-Aly HE, Tewfike TA, Ashry NM. Isolation and characterization of endophytic bacteria isolated from legumes and non-legumes plants in Egypt. Journal of Pure and Applied Microbiology,201610(1):277-290.
19. Fernandes-Salomao TM, Amorim VM, Chaves-Alves JLC, Coelho DO, Araujo EF. Isolation of pectinase hyperproducing mutants of Penicillium expansum. Rev. Microbiol, 1996:27:15-18.
20. Shrivastava UP, Kumar A. A simple and rapid plate assay for the screening of Indole-3-Acetic Acid (IAA) producing microorganism, 2011.
21. Özogul F, Özogul Y. The ability of biogenic amines and ammonia production by single bacterial cultures. European Food Research and Technology,2007:225(3):385-394.
22. Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Berg G. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and environmental microbiology,2001:67(10):4742-4751.
23. Xun W, Ren Y, Yan H, Ma A, Liu Z, Wang L *et al*. Sustained Inhibition of Maize Seed-Borne Fusarium Using a Bacillus-Dominated Rhizospheric Stable Core Microbiota with Unique Cooperative Patterns. Advanced Science, 2022, 2205215.
24. Santos AA et al. Antioxidant response of cowpea co-inoculated with plant growth-promoting bacteria under salt stress. Braz. J Microbiol,2018:49:513-521.
25. dos Santos SRL, Costa RM, de A *viz* RO, Melo VMM, de Almeida Lopes AC, de Araujo Pereira AP, Araujo ASF. Differential plant growth-promoting rhizobacteria species selection by maize, cowpea, and lima bean. Rhizosphere,2022:24:100626.
26. Kour D, Rana KL, Yadav N, Yadav AN, Kumar A, Meena VS, Saxena AK. Rhizospheric microbiomes: biodiversity, mechanisms of plant growth promotion, and biotechnological applications for sustainable agriculture. In Plant Growth Promoting Rhizobacteria for Agricultural Sustainability,2019, 19-65. Springer, Singapore.
27. Ghodsalavi B, Ahmadzadeh M, Soleimani M, Madloo PB, Taghizad-Farid R. Isolation and characterization of rhizobacteria and their effects on root extracts of Valeriana officinalis. Australian Journal of Crop Science,2013:7(3):338-344.
28. de Castro Pires R, dos Reis Junior FB, Zilli JE, Fischer D, Hofmann A, James EK, Simon MF. Soil characteristics determine the rhizobia in association with different species of Mimosa in central Brazil. Plant and soil,2018:423(1):411-428.
29. Shao J, Miao Y, Liu K, Ren Y, Xu Z, Zhang N, Xun W. Rhizosphere microbiome assembly involves seed-borne bacteria in compensatory phosphate solubilization. Soil Biology and Biochemistry,2021:159:108273.
30. Zhang J, Ma Y, Yu H. Arthrobacter cupressi sp. nov., an actinomycete isolated from the rhizosphere soil of Cupressus sempervirens. International journal of systematic and evolutionary microbiology,2012:62(11):2731-2736.
31. Bhattacharyya C, Banerjee S, Acharya U. Evaluation of Plant Growth Promotion Properties and Induction of Antioxidative Defense Mechanism by Tea Rhizobacteria of Darjeeling, India. Scientific Reports,2020:10:15536. https://doi.org/10.1038/s41598-020-72439-z.
32. Bhadrecha P, Bala M, Khasa YP. Hippophae rhamnoides L. Rhizobacteria Exhibit Diversified Cellulase and Pectinase Activities. Physiology and Molecular Biology of Plants,2020:26:1075-1085. https://doi.org/10.1007/s12298-020-00778-2.
33. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil Beneficial Bacteria and their Role in Plant Growth Promotion: A Review. Annals of Microbiology,2010:60(4):579-598. doi:10.1007/s13213-010-0117-1.
34. Khatoon Z, Huang S, Rafique M, Fakhar A, Kamran MA, Santoyo G. Unlocking the Potential of Plant Growth-Promoting Rhizobacteria on Soil Health and the Sustainability of Agricultural Systems. Journal of Environmental Management,202:273:111-118. doi:10.1016/j.jenvman.2020.111118.
35. Yadav J, Verma JP, Tiwari KN. Effect of Plant Growth Promoting Rhizobacteria on Seed Germination and Plant Growth of Chickpea (Cicer arietinum L.) under *in vitro* Conditions. Biological Forum,2010:2(2):15-18.
36. Singh D, Geat N, Rajawat MVS. Prospecting Endophytes from Different Fe or Zn Accumulating Wheat Genotypes for their Influence as Inoculants on Plant Growth, Yield, and Micronutrient Content. Annals of Microbiology,2018:68:815-833. https://doi.org/10.1007/s13213-018-1388-1.
37. Fouda A, Eid AM, Elsaied A, El-Belely EF, Barghoth MG *et al*. Plant Growth- Promoting Endophytic Bacterial Community Inhabiting the Leaves of Pulicaria incisa (Lam.) DC Inherent to Arid Regions. Plants:2021:(10):65-76. https://doi.org/10.3390/plants10010076.
38. Alkahtani MDF, Fouda A, Attia KA, Al-Otaibi F, Eid AM, Ewais EED, Abdelaal KAA. Isolation and Characterization of Plant Growth Promoting Endophytic Bacteria from Desert Plants and Their

Application as Bioinoculants for Sustainable Agriculture. Agronomy,2020:10(9):1325. doi:10.3390/agronomy10091325.
39. Olayemi OP, Odedara OO. Screening of Endophytic Plant Growth-Promoting Bacteria Isolated from Two Nigerian Rice Varieties. Nigerian Journal of Biotechnology,2007:33(1):1-9. doi:10.4314/njb.v33i1.1.
40. Banik A, Dash GK, Swain P, Kumar U, Mukhopadhyay SK, Dangar TK. Application of Rice (Oryza sativa L.) Root Endophytic Diazotrophic Azotobacter sp. strain Avi2 (MCC 3432) can Increase Rice Yield under Green House and Field Condition. Microbiology Research,2019:219:56-65.
41. Correa-Garcia S, Armand PS, St-Arnaud M, Yergeau E. Rhizoremediation of Petroleum Hydrocarbons: A Model System for Plant Microbiome Manipulation. Microbial Biotechnology,2019:11(5):819-832. doi:10.1111/1751-7915.13303 PMID:300664642008.09.014.
42. Tang A, Haruna AO, Majid NMA, Jalloh MB. Potential PGPR Properties of Cellulolytic, Nitrogen-Fixing, Phosphate-Solubilizing Bacteria in Rehabilitated Tropical Forest Soil. Microorganisms2020:8(3):442-450.
43. Emami S, Alikhani HA, Pourbabaee AA, Etesami H, Motasharezadeh B, Sarmadian F. Consortium of Endophyte and Rhizosphere Phosphate Solubilizing Bacteria Improves Phosphorous use Efficiency in Wheat Cultivars in Phosphorus Deficient Soils. Rhizosphere,2020:(14):100196.
44. Chandra A, Chandra P, Tripathi P. Whole Genome Sequence Insight of Two Plant Growth-Promoting Bacteria (B. subtilis BS87 and B. megaterium BM89) Isolated and Characterized from Sugarcane Rhizosphere Depicting Better Crop Yield Potentiality. Microbiological Research,2010:247:126733. https://doi.org/10.1016/j.micres.2021.126733.
45. Dastager SG, Deepa CK, Pandey A. Isolation and Characterization of Novel Plant Growth Promoting Micrococcus sp. NII-0909 and its Interaction with Cowpea. Plant Physiology and Biochemistry,2010:48(12):987-92. doi: 10.1016/j.plaphy.2010.09.006. Epub 2010 Sep 24. PMID: 20951599.