ISSN: 2347-4688, Vol. 12, No.(2) 2024, pg. 705-713

Current Agriculture Research Journal

www.agriculturejournal.org

Molecular Characterization of Plant Growth Promoting Rhizobacteria Isolated from Nodules of *Cicer arietinum* Plant

ANAMIKA SINGH and RAKESH KUMAR VERMA*

Department of Biosciences, School of Liberal Arts and Sciences, Mody University of Science and Technology, Lakshmangarh, Sikar,(Rajasthan), India.

Abstract

Agriculture has recently prioritized Plant Growth Promoting Rhizobacteria (PGPR) due to their significance in the rhizosphere, which is an ecological unit in the biosphere. Isolating PGPR from *Cicer arietinum* (chickpea) nodules and characterizing its PGP activities was the purpose of the present study. The research showed that the specific strain could manufacture several useful chemicals, such as hydrogen cyanide (HCN), ammonia, siderophore, indole acetic acid (IAA) and nitrogenase. Furthermore, the isolate was identified as *Bacillus licheniformis* AS11 through 16s rRNA analysis, and alignment analysis showed 99% similarity with the *Bacillus licheniformis* KPA12 isolate. It's worth highlighting that, *Bacillus licheniformis* AS11 a potential PGPR, is considered a valuable asset for agriculture when it has a positive effect on plant growth. Therefore, *Bacillus licheniformis* AS11 can be a beneficial and constructive addition to the field of agriculture. These findings suggest potential applications in sustainable agriculture by improving crop yield through natural growth-promoting mechanisms and reducing reliance on chemical fertilizers.

Article History
Received: 03 April 2024
Accepted: 23 May 2024

Keywords
IAA Production; PGPR; Phylogenetic Analysis; Rhizosphere; 16srRNA identification.

Introduction

The rhizosphere, which is a fascinating soil region that surrounds plant roots, is home to a microbial community that is both diverse and complicated. This community includes bacteria, fungi, viruses, and protists.[1] Additionally, these microorganisms play an important part in the process of aiding the uptake of nutrients, defending the plant from infections, and modifying the immune system of the plant, all of which have an impact on the plant's growth and overall health. As a result of the fact that the sorts of microorganisms that are found in the rhizosphere are dependent on the species of the host plant, the interaction that occurs between the plant and its rhizosphere microbiome is an exciting and ongoing field of research. Additionally, it has been shown that certain microbes, such as PGPRs, can dramatically stimulate plant growth, making them

CONTACT Rakesh Kumar Verma rkwat4@yahoo.com Department of Biosciences, School of Liberal Arts and Sciences, Mody University of Science and Technology, Lakshmangarh, Sikar,(Rajasthan), India.

Doi: http://dx.doi.org/10.12944/CARJ.12.2.16

an alternative to chemical fertilizers that are both sustainable and friendly to the environment.[2-3] The potential benefits of understanding and harnessing the power of the rhizosphere microbiome are far-reaching, with significant implications for agriculture and environmental management.[4]

The community of PGPR is made up of a wide variety of genera, each of which plays an important part in promoting the growth and production of plants. This community includes members from various genera such as Beijerinckia, *Azospirillum*, *Rhodococcus*, *Stenotrophomonas*, *Zoogloea*, *Bacillus*, *Ochrobactrum*, *Acinetobacter*, *Derxia*, *Herbaspirillum*, *Paenobacillus*, *Klebsiella, Burkholderia*, *Pantoae, Arthrobacter*, *Gluconacetobacter, Alcaligenes*, *Enterobacter*, *Acetobacter*, *Pseudomonas, Lactobacillus, Azotobacter, Azoarcus,* and *Serratia*.[5] To improve nutrient intake and provide protection against infections, these bacteria can colonise the root surface and change the architecture of the root system. In addition, PGPR can produce chemicals that promote plant growth, such as gibberellins, cytokinins, and indole acetic acid, which further contribute to the enhancement of plant growth and development.[6] There have been several studies that have shown that plant growth-promoting rhizobacteria (PGPR) can produce beneficial chemicals. These substances include phosphates, silica, potassium, and zinc. Through the assimilation of nitrogen that is received from biological sources and the binding of iron and other micronutrients, this mechanism promotes the growth of plants while simultaneously increasing the amount of oxygen that is available in the global atmosphere. Therefore, the stimulation of aeriform biomass synthesis results in increased root development and stem elongation. This is a consequence of the stimulation. In addition, the PGPR is responsible for the production of a wide variety of plant hormones, including ethylene, auxins, cytokinin, indole acetic acid, and gibberellins, all of which play a significant part in the process of encouraging plant growth.[7-8] According to research, the production of the enzyme recognized as 1-aminocyclopropane 1-carboxylate deaminase (ACC) has the potential to increase the density and elongation of roots in crops, while simultaneously reducing the amount of ethylene that is present in the roots. Furthermore, PGPR can efficiently modify the environment of the rhizosphere, it can drive systemic resistance and increase the plant's innate capacity to survive stress9. When it comes to bioremediating salt and fluoride contamination, Plant Growth-Promoting Rhizobacteria (PGPRs) can be incredibly useful.[10] It is also known that PGPRs enhance the total response of plant cells to environmental stimuli. This triggers the immunological response and develops systemic resistance, making the plant stronger and more resistant. Priming the plant to react more effectively to possible dangers, including pests or diseases, is how PGPRs work. Reduce the use of toxic chemicals and pesticides while simultaneously improving plant health and productivity with PGPRs, which work by optimizing the plant's response to external stimuli. It has been discovered by researchers that PGPRs can boost the production of chemicals connected to defence in plants that live in their presence.[11]

The PGPRs have demonstrated their usefulness in improving the ability of plants to withstand stress. They contribute to the promotion of germination, modification of antioxidant activities, and enhancement of nutritional levels, among other advantages. These findings not only improve our comprehension of the applications of PGPR, but they also offer useful knowledge that can be put into practice to promote sustainable agriculture in soils that are contaminated by fluoride11. Two research have shown that *Bacillus* sp. can flourish in abiotic conditions. *Bacillus subtilis* ER-08 (BST) is a resilient and adaptable rhizobacterial strain that enhances plant development. This strain has demonstrated the ability to improve the development of fenugreek (*T. foenum-graecum L*.) in conditions of high salt levels and limited water availability. In addition, *Bacillus* sp. can enhance tomato development in both normal and salt-stressed conditions through the process of phosphate solubilization and the synthesis of ACC deaminase, siderophore, and IAA. The ability of tomato seedlings to withstand salt stress is correlated with higher levels of osmoregulatory proline and soluble sugar, as well as an increase in the activity of ROS-scavenging enzymes. All of these factors contribute to the plant's ability to withstand salt stress.[12-13] A total of six rhizobia-like bacterial strains were isolated from the root and stem nodules of different leguminous plants. These strains were then characterised for their capacity to promote growth in the ICCV 2 variety of chickpeas. This study proposes that the bacteria

found in the root and stem nodules of chickpea plants have the potential to improve nodulation, plant growth promotion, and agricultural yields.[14]

Materials and Methods

Following the collection of nodules from chickpeas (*Cicer arietinum*) in Lakshmangarh, Sikar (Rajasthan), the nodules were cultured on yeast extract mannitol agar (YEMA) media that was received from Himedia. The content of the YEMA medium was as follows: 1.0 grammes per litre of yeast extract, 0.5 grammes per litre of dipotassium phosphate, 10.0 grammes per litre of mannitol, 0.1 grammes per litre of sodium chloride, 0.2 grammes per litre of magnesium sulphate, 0.025 grammes per litre of Congo red, and 20.0 grammes per litre of agar. The medium was incubated at a temperature of 37ºC for a period of twenty-four hours to produce cultures that were free of contamination by the use of the streaking technique, which was carried out as many times as necessary. In the subsequent step, the distinct strains were kept at a temperature of -80 degrees Celsius in a solution that included 25% glycerol.

The bacterial isolates were inoculated on culture media for 24 hours and followed by microscopic examination. A microscope was then employed to inspect the edge, colour, elevation, and surface morphology of the specimens, and the conventional approach was utilized to gram stain the specimens following this examination. To get a qualitative estimation of the PGP activities of the selected bacterial strains, a few minor changes were made. The effectiveness of the production of the catalase enzyme in bacteria that were isolated was evaluated. In bacterial strains, the enzyme known as catalase is accountable for the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. A thin smear of the isolates was made on glass slides to carry out the test. After that, a few drops of hydrogen peroxide were added to each slide. If there was effervescence and the creation of gas bubbles, this indicated that the test was successful. Using a nutritional agar plate that had been amended and enriched with glycine at a concentration of 4.4gm/L, the isolates were streaked on the plate to ascertain the amount of HCN that had been produced. This was done to accomplish the task. After being dipped in a solution that contained sodium carbonate at a concentration of 2% and picric acid at a concentration of 0.5%, following, a piece of Whatman filter paper was positioned on the top surface of the Petri plates. This was done after the paper had made contact with the solution. The plates were first covered with parafilm, and then they were placed in an incubator at a temperature of 28 °C for four days and then removed from the incubator. It was determined that hydrogen cyanide (HCN) was present in the atmosphere when a colour that spanned from orange to red appeared. This was an indication that HCN was present.

In separate test tubes that contained 10 ml of peptone water, bacterial strains were cultivated and then administered an inoculum. Subsequently, the test tubes were incubated in an incubator at a temperature of 28°C for a duration of forty-eight hours. Each test tube received 0.5ml of Nessler's reagent once the incubation period had concluded. Indicative of the presence of ammonia generation is the emergence of a brown or yellow colour in the test tube.

Over the course of five days at a temperature of 28 °C, isolated strains were streaked on Pikovskaya's medium plates. The indication that the isolates were capable of solubilizing phosphate was provided by the appearance of a halo zone surrounding the colony.[15]

When determining the amount of IAA that was produced by isolates, the method that was provided by Singh *et al.* (2024) was utilized. Following the introduction of the bacterial cultures, a nutrient broth medium that contained an additional 1g L-1 of tryptophan was used throughout the experiment. For a period of four days, the flasks were stirred at a speed of 80 rpm at a temperature of 30˚C. Centrifugation at a speed of 4000 rpm for a period of five minutes was used to collect the particles that were isolated. Two drops of orthophosphoric acid with a concentration of 10 m and 4.0 mL of Salkowski reagent were added to the supernatant, which was 2.0 mL in volume. After being incubated at room temperature, the combination was examined. It was determined that the generation of indole-3-acetic acid (IAA) was determined by the presence of pink color. A method that was presented by Singh *et al.* (2024) was utilised to evaluate the efficiency of the generation of siderophores. Chromium azurol S (CAS) agar plates were injected with bacteria, and then they were incubated at 30˚C for 24 hours. Upon completion of the incubation period, the development

of siderophores was validated by the transformation of the medium from blue to orange in color.

Jensen's agar medium was employed to enable the detection of nitrogen-fixing bacteria capable of thriving in nitrogen-deficient conditions. Subsequently, the medium was transferred onto the sterilized petri plates. Subsequently, the isolates were centrifuged in a physiological saline solution to eliminate any residual nitrogen from the previous liquid medium. The isolated bacteria underwent two days of injection and incubation at a temperature of 28±2°C. Subsequently, the isolates that exhibited growth were streaked on Jensen's agar media to ascertain their capacity to perform nitrogen fixation.[16]

The isolates selected for this investigation were characterized by employing 16S rRNA sequencing following the methods described in Mishra *et al.* (2022) with slight modifications. With some minor adjustments, the phenol-chloroform-isoamyl alcohol (PCI) method was utilised to extract the genomic DNA. A nano spectrophotometer (Eppendorf Bio spectrometer), was utilized to assess both the quantity and quality of the DNA that was isolated. The 16S rRNA gene was amplified using standard parameters in a thermal cycler. Finally, the PCR products were sequenced in both the forward and reverse directions employing the forward primer 5'-GGATGAGCCCGGCCTA-3' and the reverse primer 5'-CGGTGTGTACAAGGCCCGG-3'.

To understand the evolutionary ancestry of the species being studied, we utilized the JC (Jukes-Cantor model) and the ML approach. To represent this historical data, we constructed a Bootstrap consensus tree using 1000 iterations. The Neighbor-Join and Bio-NJ algorithms were utilised to generate initial trees for the heuristic search. These trees were constructed with the help of a matrix of pairwise distances that was computed with the Jukes-Cantor model. According to the log probability value, we chose the topology that had the highest value. A JC substitution model and 1000 bootstrap values were utilized in the performance of the tests, which were carried out in the MEGA11 software.[17-18]

Results

Characterization and PGP Activities of Rhizobacteria

The samples that were collected from chickpea (*Cicer arietinum*) plants were used to generate a pure culture of plant growth-promoting rhizobacteria (PGPRs). After that, these rhizobacteria were examined to determine their characteristics, as shown in Table 1. During the course of the inquiry, it was discovered that the nodules of the chickpea plants contained rhizobacteria. This finding suggests that these microbes are capable of developing symbiotic connections with the plant. The isolate exhibited remarkable characteristics, including the capacity to synthesize indole-3-acetic acid (IAA), a widely recognized phytohormone involved in the growth and development of plants. The synthesis of IAA by rhizobacteria primarily impacts the root system by augmenting its dimensions, mass, quantity of lateral roots, and the surface area in contact with the soil. This technique enhances nutrient exploration and uptake in soil, hence enhancing plant growth and productivity. Furthermore, it showcased the production of ammonia, which aids in enriching the nitrogen levels in the soil, and it showcased the synthesis of siderophores, which are essential for extracting iron from the soil. In addition to this, the isolate demonstrated the capacity to produce hydrogen cyanide (HCN), which is a compound that is typically associated with antibacterial capabilities. In this context, HCN does not function as a biocontrol agent, but instead plays a role in geochemical processes within the substrate, such as the chelation of metals. By means of an indirect process, the accessibility of phosphate is enhanced. It also can solubilize phosphate, which is a process that helps to increase the amount of phosphorus that is available to the plant. Plant-associated bacteria play a significant role in promoting plant growth through the mechanism of inorganic phosphate solubilization. This process entails the secretion of organic acids by bacteria into the soil, which causes the phosphate complexes to dissolve and transform into ortho-phosphate. This form of phosphate is then accessible for plants to absorb and utilize. These abilities suggest that these microbes could potentially enhance the plant's growth and resistance to pathogens. The isolate was positive for catalase and nitrogenase measurements, which are enzymes involved in plant growth and nitrogen fixation. These findings offer novel perspectives on the possible advantages of these microbes in enhancing plant growth and present promising prospects for future study in this field.

Table 1: Below is the data for the primary and secondary screening of the isolate. The isolate was obtained from the nodules of chickpea (*Cicer arietinum*). It includes Gram Staining, Colony morphology, and qualitative estimation of PGP activities.

Code	Primary Screening of isolates		Secondary Screening of the isolates						
	Gram Staining	Colony	NH_4	HCN	Sidero-	IAA	PO_4	Catalase	Nitrogenase
	+/-	Morphology			phore				
1	+	Rod shape, rough, opaque, off white	+	+	++	++	+	+	+

Table 2:Species identification of the rhizobacterial isolate.

Code	Accession no.	Species name	Similarity with other species	% Similarity
1.	PP038143	*Bacillus licheniformis* AS11	*Bacillus licheniformis* KPA12	99%
			Bacillus subtilis UYY	100%
			Bacillus subtilis MT37	100%
			Bacillus subtilis stercoris strain SM-7	100%
			Bacillus subtilis strain CILBBTN607105190	100%

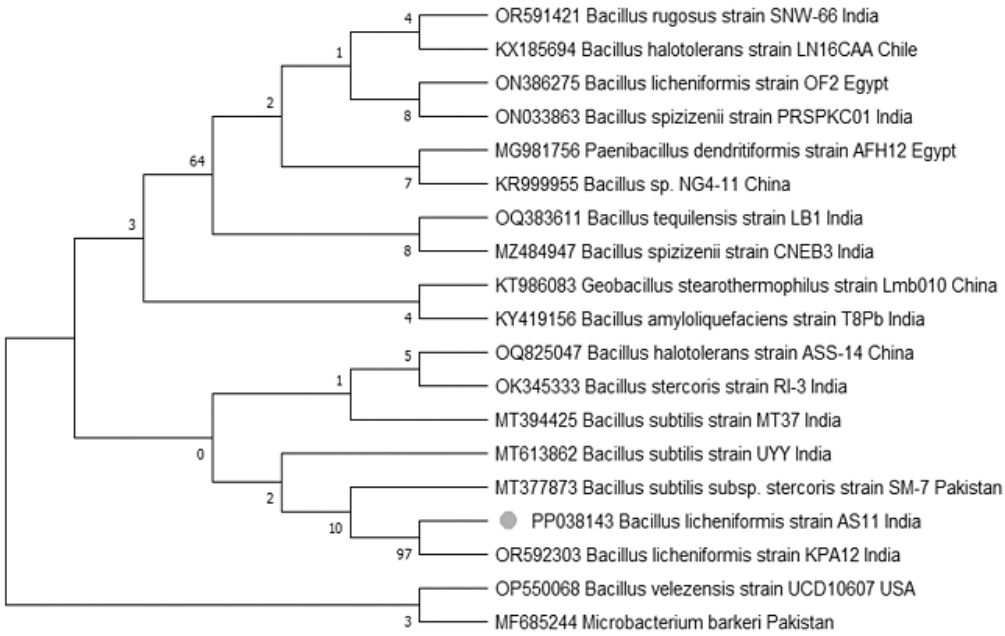

Fig. 1: The phylogenetic tree of isolate no. 1 *Bacillus licheniformis* AS11 and its common ancestry with other geographical isolates

Molecular Characterization of the isolates

The rhizobacterial isolates were thoroughly analyzed using 16S rRNA, and the strains were identified. Their respective accession numbers, listed in Table 2, have been deposited in NCBI GenBank.

Based on the data presented in the figure, it can be observed that Isolate No. 1, which is the *Bacillus licheniformis* AS11, shares a higher similarity with another type of *Bacillus licheniformis*, specifically the KPA12 variant. The information is depicted in Figure 1, which presents a visual illustration of the relationship between the two isolates.

Discussion

In this study, the researchers sought to understand the PGPRs present in the nodules of chickpea (*Cicer arietinum*), a crucial crop in many parts of the world. To do so, the PGPR was isolated and identified by using 16S rRNA gene sequencing. The analysis exhibited that the PGPR isolates belonged to the *Bacillus genus*, which is known to exhibit plant growth promotion (PGP) characteristics. This in vitro analysis was carried out to determine the PGP properties of the PGPR that was isolated. In addition to being an essential component of plant and soil ecology, these PGPRs are also an essential component in the process of preserving the equilibrium of the soil microbiome. After gaining a grasp of the PGP features of these *Bacillus* PGPRs, researchers will have a better understanding of how to encourage plant growth and preserve the health of the soil to achieve sustainable agriculture. The PGPRs are an essential component of the ecosystem that includes both plants and soil. They play a significant part in ensuring that the soil microbiome remains in a state of equilibrium. *Bacillus subtilis, Bacillus pumilus,* and *Bacillus cereus* are all examples of *Bacillus* species that display characteristics of the PGP bacteria that help plants grow by producing phytohormones like IAA and gibberellic acid, as well as enzymes like ACC deaminase, chitosanase, protease, glucanase, and cellulase. They also synthesize compounds such as HCN, biofilm preparation, and lipopeptides.[19-21] *Bacillus* is a type of bacteria that helps to produce organic acids in soil. These organic acids are important because they release phosphorus (P) to plants, which is essential for their growth. In addition, *Bacillus* also increases the activity of acid phosphatases, which further promotes plant growth. A study conducted in 2018 by Saeid *et al.* confirmed that *B. cereus*, *B. megaterium* and *B. subtilis* are particularly effective at improving P solubilization. It is because these bacteria are responsible for the production of organic acids, which include gluconic acid, acetic acid, succinic acid, lactic acid, and propionic acids. These acids are essential in the process of increasing the solubilization of phosphorus in soil.[22] Bhardwaj *et al.* 2023 suggested that rhizobacterial strains have the potential to be utilized to inoculate the soil to boost plant growth by synthesizing HCN[23]. The ability of *Bacillus* strains to produce siderophores is thus a valuable trait that can enhance plant growth and development.[8] The study also found that *Bacillus* bacteria strains have several properties that promote plant growth and increase crop yields.[24] Therefore, these bacteria strains could be used in the agricultural industry to enhance plant growth and yields.[25-26]

The purpose of conducting PGP tests was to identify the strain of rhizobacterial species that have the most potential for promoting plant growth. In this study, the isolate showed remarkable abilities, including the capability to generate IAA, which is a well-known phytohormone that is involved in plant growth. It also exhibited siderophore synthesis, which is essential for obtaining iron from the soil, and showed ammonia production, which helps to improve the soil's nitrogen content. Moreover, the isolate showed the ability to generate hydrogen cyanide (HCN), which is known to have antimicrobial properties. It was also capable of phosphate solubilization, which helps to make phosphorus more available to the plant. These abilities suggest that these microbes could potentially enhance the plant's growth and resistance to pathogens. The isolate was positive for catalase and nitrogenase measurements, which are enzymes involved in plant growth and nitrogen fixation. Additionally, the isolate was identified using 16S rRNA sequencing. This study will help to accurately identify rhizobacterial isolates that possess essential traits for controlling plant growth and enhancing production. The present study has revealed promising results that strains of *Bacillus* bacteria possess significant plant growth-promoting properties, as demonstrated in in-vitro tests. These findings suggest the potential of utilizing

Bacillus bacteria in agriculture to enhance plant growth and improve crop yields, owing to their diverse plant growth-promoting properties. Implementing PGPRs as a biofertilizer can be a constructive approach to significantly improve plant growth and overall productivity. Further research on rhizobacterial isolates can help us better understand the mechanisms that support plant growth and establish their use as effective bioinoculants across different types of soils.

Conclusion

The study aimed to identify rhizobacterial strains with potential for promoting plant growth. The isolate showed significant abilities, including the ability to generate phytohormones, siderophore synthesis, ammonia production, hydrogen cyanide (HCN), and phosphate solubilization. These abilities suggest that these microbes could enhance plant growth and resistance to pathogens. The isolate was identified using 16S rRNA sequencing and showed positive for catalase and nitrogenase measurements. The findings suggest the potential of using *Bacillus licheniformis* AS11 bacteria in agriculture to enhance plant growth and improve crop yields. Implementing PGPRs as biofertilizers can significantly improve plant growth and productivity. Further research on rhizobacterial isolates can help understand their mechanisms and establish their use as effective bioinoculants across different soil types.

Acknowledgement

The authors would like to express their gratitude to the Department of Biosciences at Mody University of Science and Technology in Lakshmangarh, Sikar, Rajasthan (SM/2020-21/008 and SM/2022-23/008) as well as the Department of Science and Technology in New Delhi (DST/CURIE-01/2023/MU) for providing financial assistance for the scientific study.

Funding Sources

Department of Biosciences at Mody University of Science and Technology in Lakshmangarh, Sikar, Rajasthan (SM/2020-21/008 and SM/2022-23/008) and Department of Science and Technology in New Delhi (DST/CURIE-01/2023/MU).

Conflict of Interest

No conflict of Interest

Data Availability Statement

The study includes the original contributions, which may be found in the article and accessible through the NCBI gene bank using the accession number of the deposited sequence. For any additional questions, please contact the corresponding author

Ethics Statement

Not applicable

Authors' Contribution

Both AS and RKV contributed to the conceptualization of the work that was being done. RKV went through the manuscript and made his final revisions. All authors have read and agreed to the published version of the manuscript.

References

1. Leach JE, Triplett LR, Argueso CT, Trivedi P. Communication in the phytobiome. *Cell.* 2017;169(4):587-596. doi:10.1016/j.cell.2017.04.025
2. Ling N, Wang T, Kuzyakov Y. Rhizosphere bacteriome structure and functions. *Nature Communications.* 2022;13(1). doi:10.1038/s41467-022-28448-9
3. Choudhary N, Dhingra N, Gacem A, *et al.* Towards further understanding the applications of endophytes: Enriched source of bioactive compounds and bio factories for nanoparticles. *Frontiers in Plant Science.* 2023a;14. doi:10.3389/fpls.2023.1193573
4. Mahanty T, Bhattacharjee S, Goswami M, *et al.* Biofertilizers: A potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*. 2016;24(4):3315-3335. doi:10.1007/s11356-016-8104-0
5. Vega-Celedón P, Bravo G, Velásquez A, *et al.* Microbial diversity of psychrotolerant

bacteria isolated from Wild Flora of Andes Mountains and Patagonia of chile towards the selection of plant growth-promoting bacterial consortia to alleviate cold stress in plants. Microorganisms. 2021;9(3):538. doi:10.3390/*microorganisms*9030538

6. Backer R, Rokem JS, Ilangumaran G, *et al.* Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of Bio-stimulants for Sustainable Agriculture. *Frontiers in Plant Science.* 2018;9. doi:10.3389/fpls.2018.01473
7. Constantia J, Ferniah RS. Vegetative growth of Rainbow Chili (*Capsicum annuum* L.) in the treatment of PGPR (plant growth promoting rhizobacteria), PGPR-NPK fertilizer, and PGPR-compost combination. *Agric.* 2020;32(2):95-104. doi:10.24246/agric.2020.v32.i2.p95-104
8. Choudhary N, Rajvanshi K, Gaur RK, Verma RK.. Molecular Characterization and Plant Growth Promotion Potential of Endophyte Isolated from Desert Plant. *Annals of. Agri Bio Research.* 2023b ;28 (2): 232-238.
9. Tiwari G, Duraivadivel P, Sharma S, P. H. 1-aminocyclopropane-1-carboxylic acid deaminase producing beneficial rhizobacteria ameliorate the biomass characters of *Panicum Maximum Jacq.* by mitigating drought and Salt *Stress. Scientific Reports.* 2018;8(1). doi:10.1038/s41598-018-35565-3
10. Singh A, Yadav VK, Gautam H, *et al.* The role of plant growth promoting rhizobacteria in strengthening plant resistance to fluoride toxicity: A review. *Frontiers in Microbiology.* 2023;14. doi:10.3389/fmicb.2023.1271034
11. Singh A, Patani A, Patel M, *et al.* Tomato seed bio-priming with *Pseudomonas aeruginosa* strain PAR: A study on plant growth parameters under sodium fluoride stress. *Frontiers in Microbiology.* 2024;14. doi:10.3389/fmicb.2023.1330071
12. Patani A, Prajapati D, Ali D, *et al.* Evaluation of the growth-inducing efficacy of various *bacillus* species on the salt-stressed tomato (*Lycopersicon esculentum mill.*). Frontiers in Plant Science. 2023;14. doi:10.3389/fpls.2023.1168155
13. Patel M, Islam S, Husain FM, *et al. Bacillus subtilis* ER-08, a multifunctional plant growth-promoting rhizobacterium, promotes the growth of fenugreek (*Trigonella foenum-Graecum* L.) plants under salt and drought stress. Frontiers in Microbiology. 2023;14. doi:10.3389/fmicb.2023.1208743
14. Mir, Mohammad Imran *et al.* "Characterization of rhizobia isolated from leguminous plants and their impact on the growth of ICCV 2 variety of chickpea (*Cicer arietinum* L.)." Heliyon 7 (2021): n. pag. https://doi.org/10.1016/j.heliyon.2021.e08321
15. Bhattacharyya C, Bakshi U, Mallick I, Mukherji S, Bera B, Ghosh A. Genome-guided insights into the plant growth promotion capabilities of the physiologically versatile *bacillus aryabhattai strain* AB211. Frontiers in *Microbiology.* 2017;8. doi:10.3389/fmicb.2017.00411
16. Vishwakarma D, Thakur JK, Gupta SC. A study on nitrogen fixing ability of soil isolates and quantitative estimation of K solubilization by soil and plant isolates. *International Journal of Current Microbiology and Applied Sciences.* 2017;6(12):2641-2647. doi:10.20546/ijcmas.2017.612.305
17. Mishra M, Verma RK, Pandey V, *et al.* Role of diversity and recombination in the emergence of Chilli Leaf Curl virus. *Pathogens*. 2022;11(5):529. doi:10.3390/pathogens11050529
18. Mishra R, Verma RK, Mall S, Gaur RK. Complete genome sequence of eggplant mild leaf mottle virus (EMLMV) infecting eggplant in India. Indian *Phytopathology.* 2023;76(4):1141-1144. doi:10.1007/s42360-023-00672-3
19. Mahapatra S, Yadav R, Ramakrishna W. *Bacillus subtilis* impact on plant growth, Soil Health and Environment: Dr. Jekyll and mr. Hyde. *Journal of Applied Microbiology.* 2022;132(5):3543-3562. doi:10.1111/jam.15480
20. Tahir HA, Gu Q, Wu H, *et al.* Plant growth promotion by volatile organic compounds produced by *bacillus subtilis* SYST2. *Frontiers in Microbiology.* 2017;8. doi:10.3389/fmicb.2017.00171
21. Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ. *Bacillus* species in soil as a natural resource for plant health and Nutrition. *Journal of Applied Microbiology.*

2019;128(6):1583-1594. doi:10.1111/jam.14506

22. Saeid A, Prochownik E, Dobrowolska-Iwanek J. Phosphorus solubilization by *bacillus* species. *Molecules.* 2018;23(11):2897. doi:10.3390/molecules23112897
23. Bhardwaj P, Chauhan A, Ranjan A, *et al.* Assessing growth-promoting activity of bacteria isolated from municipal waste compost on Solanum lycopersicum L. *Horticulturae.* 2023;9(2):214. doi:10.3390/horticulturae9020214
24. Ilyas T, Vishwakarma SK, Shahid M, Malviya D, Kumar S, Singh S, *et al.* Exploring the potentiality of *bacillus amyloliquefaciens* as a prominent Biocontrol Agent: A comprehensive overview. Microorganisms for Sustainability 2024:133–50. doi:10.1007/978-981-99-8195-3_7.
25. Luo L, Zhao C, Wang E, Raza A, Yin C. *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: An overview for its mechanisms. Microbiological Research 2022;259:127016. doi:10.1016/j.micres.2022.127016.
26. Vlajkov V. Potential of *Bacillus Amyloliquefaciens* as a Biocontrol Agent Produced on Different Carbon Source-Based Cultivation Media 2020. doi:10.26226/morressier.5f97e7baaee6041e8a666201.