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Characterization of Plant Growth-Promoting *Bacillus* Isolates from the Rhizosphere of Tomatoes using Molecular and Functional Techniques

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Abstract - The rhizosphere, a critical environment that harbors a wide variety of microorganisms, is indispensable for facilitating plant development and maintaining soil quality. Bacillus genus members, referred to as Plant Growth Promoting Rhizobacteria (PGPR), possess the capacity to substantially enhance agricultural productivity and promote long-term sustainability. This study focuses on the methodology employed to isolate and characterize seven Bacillus PGPR (TRS-1-8) isolates that were identified in the rhizosphere of tomato plants. An assortment of characteristics that promote plant growth were evaluated in the PGPR strains: solubility in zinc and phosphate, synthesis of phytotoxins and siderophores, chitinase production, hydrogen cyanide (HCN) and indoleacetic acid (IAA) synthesis, biofilm formation, and ACC deaminase activity. Furthermore, an assessment was conducted on their ability to impede the growth of pathogenic bacteria and fungi that have an adverse effect on plants. The identification of the Bacillus spp. in these isolates was achieved via genetic characterization utilizing 16S rRNA gene sequencing. In order to verify the genetic diversity of the isolates, BOX-PCR fingerprinting was an additional technique employed. The efficacy with which the isolates established colonies on tomato roots was observed. The results obtained from the greenhouse experiments demonstrated that the chosen isolates had a beneficial effect on a range of growth parameters pertaining to tomato plants. These parameters comprised shoot length, root length, and dried weight. The statistical analyses were conducted using GraphPad Prism, where the significance of the observed differences was emphasized. In summary, the Bacillus PGPR isolates obtained from the rhizosphere of the tomato plant demonstrate a wide range of growth-promoting attributes that make them highly beneficial for the implementation of sustainable agricultural methods. The study makes substantial advancements in the comprehension of the potential applications of these techniques in the fields of biofertilization and plant health management.

Keywords - Rhizosphere, Bacillus, Plant Growth Promoting Rhizobacteria (PGPR), 16S rRNA gene sequencing, BOX-PCR Fingerprinting, Tomato Plants

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INTRODUCTION

The rhizosphere is home to a multitude of advantageous microorganisms that promote vegetable growth. Naturally occurring microorganisms possess the capability to improve soil health, stimulate plant growth, and facilitate development. The mutualistic associations that develop between microorganisms and roots in the rhizosphere have a substantial influence on both plants' overall health and soil productivity (Akinrinlola et al., 2018). These interactions effectively regulate a wide range of physical and chemical processes occurring within the soil. Plant Growth Promoting Rhizobacteria (PGPR) encompass a diverse array of bacteria that cultivate in the rhizosphere. (Shah et al., 2020) The substances produced by microorganism's aid in the development of plants and provide them with numerous functions. Crop protection against phytopathogens and encouragement of plant growth are essential components of the PGPR inoculation method. According to Mumtaz et al. (2017), it operates as an ecologically sustainable alternative to conventional fertilizers and fungicides. Examining the spectrum of potential PGPR strains that are compatible with diverse soil types and climatic

conditions is therefore crucial for the advancement of sustainable agriculture.

Bacillus is an exceptionally ubiquitous and genetically diverse genus of Plant Growth-Promoting Rhizobacteria (PGPR) that are simple to cultivate (del Carman Orozco-Mosqueda et al., 2020). Bacilli are an indispensable instrument for increasing agricultural output and efficiency due to their exceptional plant growth promotion (PGP) characteristics and extraordinary rhizosphere colonization capabilities. This phenomenon can be attributed to their capacity to stimulate the development of plants. Multiple mechanisms, including electron sorption of atmospheric nitrogen into a usable state, siderophores-mediated iron capture, and phytohormone production, are utilized by *Bacillus* species to promote development (Helal et al., 2022). One of the mechanisms by which bacteria confer beneficial effects on plants is through the production of ACC deaminase, an enzyme that is involved in the catabolism of ethylene. Additional mechanisms encompass antibiosis, detoxification, the generation of lytic enzymes, and the removal of virulence components of pathogens. Seed bacterization is a frequently employed method for investigating the impact of *Bacilli* or their preparations on plant development processes. The potential benefits of employing Beneficial *Bacillus* species as external inputs in agriculture include improved soil health and increased agricultural productivity, as stated by Sansinen (2019).

Globally, the tomato, which is formally referred to as *Solanum lycopersicum* L., is an exceptionally popular vegetable. In order to improve the yield, quality, and safety of tomatoes, it is imperative to adopt non-chemical alternatives (Sharma et al., 2015). Substantial research has been devoted to the investigation of biofertilizers containing *Bacillus* PGPR in an effort to enhance tomato yield and fruit quality. Rocha et al. (2017) identified several species, including *Bacillus licheniformis, Bacillus subtilis, Bacillus polymyxa, Bacillus cereus, Bacillus amyloliquefaciens, Megaterium, and Pumilus*, which effectively colonize the rhizosphere of the tomato plant, thereby enhancing its growth and productivity.

In the realm of molecular-level bacterial species identification and classification, BOX-PCR fingerprinting and 16S rRNA gene sequencing are frequently employed. This procedure incorporates the amplification of BOX-elements, which are repetitive DNA sequences potentially identified in the genomes of additional organisms, via the utilization of the BOX-A1R primer. This exemplifies the diversity present within a given species. This research paper presents an examination of various PGPR (Plant Growth Promoting Rhizobacteria) that have been identified in the rhizosphere of tomato plants under both laboratory and natural conditions (Castaldi et al., 2021).

MATERIAL AND METHODS

- **Seed Material:** The seeds of the tomato plant, also known as Solanum lycopersicum L., were acquired from the Agriculture Research Institute in Mithapur, Patna, Bihar. More precisely, the seeds of the Arka Vikas diversity were obtained.

- **Microbial Cultures:** Indian Academy of Biotechnology, SIMTECH College, Patna, Bihar supplied the CBli strain of *Bacillus licheniformis*. Indian Institute of Technology, Patna supplied the fungal infections, specifically *Fusarium* sp. and *Curvularia* sp. From the RMRIMS Agamkuan, Patna, Bihar, India, the bacterium *Xanthomonas oryzae* pv. oryzae (Xoo) strain BXO43, which causes plant diseases, was obtained. From our laboratory collection originates the *Xanthomonas axonopodis* pv. citri (Xac) strain.

- **Collection of rhizospheric soil samples:** Tomato plants in good health were removed from the ground, and samples of soil were gathered from the area around their roots at many tomato farms located in different districts of Bihar, India. Antiseptic containers were used to collect and conserve soil samples from the rhizosphere, keeping them at a temperature of 4oC until they were needed for further processes (Saqib et al., 2020).

- **Isolation of rhizobacteria:** To identify rhizobacteria, a mixture of one gram of rhizosphere soil and ten milliliters of phosphate buffer saline (PBS) at a pH of 7.0 was prepared. Following many diluting and vortexing steps, the liquid was plated onto two distinct kinds of media: The following components make up minimum medium-1 (M1), expressed in grams per liter: Following that, the plates were maintained at 30 ◦C for 48 hours in an incubator. After being transferred to a fresh growth medium, the isolates that exhibited distinctive characteristics underwent purification. Five bacterial isolates (TRS-1-8) were assessed utilizing CBli in this investigation.

- **Rhizobacterial isolates are chosen according to their capacity to promote plant growth:** The assessment of the rhizobacterial isolates' in vitro Plant Growth-Promoting (PGP) properties was conducted using established testing protocols. This study integrated assessments of enzyme production and biofilm formation, encompassing soluble zinc and phosphate, siderophore, and ACC deaminase measurements (Scagliola et al., 2016). The growth inhibition susceptibility of the isolates was evaluated using soil-dwelling phytopathogenic fungi Fusarium sp. and Curvularia sp., as well as Xanthomonas phytopathogenic strains Xac and Xoo.

- **Characterization of rhizobacterial isolates:** Standard methodologies were employed to perform the physiological and biochemical characterization. The identification of seven selected isolates was performed at the genus level through the utilization of 16S rRNA gene sequencing. BOX-PCR was employed to augment the differentiation between species and sub-species.

- **Molecular characterization of rhizobacteria using 16S rRNA genes:** Isolates of rhizobacteria were cultured for a duration of 12 hours at a temperature of 30°C and a stirring rate of 160 grams in broths designated M1 and M2. Extracting information from genomic DNA. Purification, electrophoresis, and sequencing of the PCR outcomes followed. The PCR products of the amplification of the 16S rRNA gene using universal primers (27F and 1494R) were analyzed for sequence. Utilizing the BLAST program, nucleotide sequences were analyzed at the National Center for Biotechnology Information (NCBI) to determine the identity of rhizobacterial isolates according to their percentage of similarity. An accession number was allocated to each 16S rDNA sequence subsequent to its inclusion in GenBank. The utilization of the MEGA6 program to align all sequences facilitated the construction of a phylogenetic tree, according to research published by Amaresan et al., 2019.

- **Analysis for BOX-PCR:** Following the recommended protocol, BOX-PCR was used to analyze the genomes of closely related Bacillus strains using the BOX-A1R primer (50-CATA-CGGCAAGGCGACGCT-30). Thirty cycles make up a polymerase chain reaction (PCR). The first denaturation phase, which lasts for five minutes at 95 °C, comes first. The reaction is then subjected to one minute of 94 ◦C denaturation, one minute of 50 ◦C annealing, and one minute of 72 ◦C polymerization. Finally, it concludes with a last extension lasting 10 minutes at 72 °C. The amplicons were separated electrophoretically on a 2% TAE-agarose gel.

- **Growth of plants in tissue culture:** Tomato seeds were sterilized on the surface using a solution of sodium hypochlorite with a concentration of 2%. Following that, bacterial cultures with 1 x 108 colony-forming units (CFU) per milliliter (mL) were placed in a sterile carboxymethyl cellulose (CMC) solution at a concentration of 1% on these seeds. Utilized as the positive and negative controls were CBli and CMC, respectively (Dar et al., 2018). Immediately after the blotting process, the seeds that had been treated were placed to plant tissue culture bottles that were filled with half-strength Murashige and Skoog (MS) medium that had been supplied by the Indian Business Hi-media. During the time that these bottles were being preserved, they were maintained in a controlled setting that consisted of a plant growth chamber environment. The chamber was heated to a temperature of 26 degrees Celsius, and a photoperiod consisting of 16 hours of light and 8 hours of darkness was used. Before the experiment started, the light intensity was also changed to 40 micromoles per square meter per second. After the bottles were placed in this environment, 45 days had passed. Throughout this time, the bottles were ignored. Four iterations of this experiment were conducted, using three sets of identical samples for each iteration. After fifteen days, three seedlings were randomly selected from each replication, and their shoot and root lengths were measured. The samples were first dried in an oven until a consistent weight was obtained in order to calculate the dry masses of the samples (Jangir et al., 2018).

After growing seedlings on MS media for 5, 10, and 15 days, the depth of root colonization was assessed by collecting the seedlings. Following their removal, the roots were diluted in a 0.85% saline solution and then spread out on agar plates labeled M1 and M2. Following that, the dishes were incubated at 30 degrees Celsius for 24 to 48 hours. Counting the colony-forming units (CFUs) allowed for the determination of the number of colonies after incubation (Kalam et al., 2020).

- **Plant growth in greenhouse:** During the greenhouse container investigations, certain rhizobacterial isolates (TRS-2, 7, and 8) were used in conjunction with control groups (CBli and CMC). Prior to the beginning of the experiment, a Soil Test Kit that was manufactured in India by Hi-Media was used in order to determine the physicochemical properties of the soil that was present in the greenhouse. There was a surface sterilization of the tomato seedlings, followed by an injection of microorganisms, and finally, the seedlings were put in plastic pots that were filled with greenhouse soil. For the purpose of facilitating the plants' development, they were cultivated in the controlled environment of a greenhouse. A total of sixteen hours of light and eight hours of darkness were determined to have occurred under these conditions. Additionally, the relative humidity was set at 70%, and the temperature was maintained at 30 ± 2°C. The plants were irrigated daily with an equivalent volume of municipal water, devoid of any supplementary nutrients or PGPR inoculation. Three identical sets of samples were utilized to conduct the experiment a total of three times. The dry weight and root and shoot length, among other plant growth indicators, were evaluated at the 20, 40, and 60-day marks subsequent to inoculation. Three plants were selected at random and eliminated from each treatment in order to accomplish this (Chauhan et al., 2014).

- **Data analysis:** It was determined whether or not

there were statistically significant differences in the means by doing a statistical analysis on the data using the GraphPad Prism program (Version 6.0). There was an ANOVA, which may have been a one-way or a two-way analysis. Next, a post hoc test was used to compare different means; the specific test employed depended on the situation but was often Dunnett's or Bonferroni's. The Dunnett's test was used for the purpose of comparing the control mean while doing multiple comparisons, whilst the Bonferroni's test was utilized for the purpose of conducting pairwise comparisons. To determine whether statistical significance was reached, the authors utilized a threshold alpha level of 0.05 (Islam et al., 2019).

RESULTS

- **Rhizobacteria isolation, selection, and characterization:** The tomato rhizosphere yielded sixty different bacterial colonies, which were isolated using standard plating techniques on two minimal media. Tomato rhizosphere (TRS) isolates were assigned unique suffixes to seven of the sampled isolates based on their unusual colony morphology. Stated differently, M2 acquired TRS-2, TRS-4, TRS-7, and TRS-8, whereas M1 isolated TRS-1, TRS-3, and TRS-5. Table 1 provides specifics on the physiological and biochemical characteristics of these bacterial strains.

Table 1: Evaluation of the rhizobacterial isolates' physiological and biochemical properties (Kalam et al., 2020)

Attributes	TRS-1	TRS-2	TRS-3	TRS-4	TRS-5	TRS-7	TRS-8
Temperature Optima range (oC)	30 (30–40)	30 (28–40)	30 (35–40)	30 (28–45)	30 (37–45)	30 (30–40)	30 (28–40)
pH Optima range	7.0 (6.5–8.0)	7.0 (6.5–8.0)	6.0 (7.0–8.5)	7.0 (5.0–8.0)	7.0 (6.0–8.0)	7.0 (7.0–8.5)	7.0 (7.0–8.5)
Nature	Optional Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Motility	Motile	Motile	Non-Motile	Motile	Motile	Non-Motile	Non-Motile
Gram Staining	+	+	+	+	+	+	+
BIOCHEMICAL TESTS							
Voges-Proskauer Test	+	+	+	+	+	+	+
Utilization of Glucose	+	-	-	+	+	+	-
Test for production of H2S	+	-	-	-	+	+	-
Methyl red Test		-	-	-	+	+	+
Indole Test	-	+	-	-	-	+	-
Hydrolysis of Starch	-	-	+	+	+	-	+
Catalase test	+	+	+	-	-	-	+

The rhizobacterial isolates were subjected to physiological and biochemical characterization under standardized circumstances. A positive result was denoted by '+' whereas a negative result was denoted by '-' for each test.

- **PGP characteristics exhibited by rhizobacterial isolates:** In accordance with the data shown in Table 2, the seven Gram-positive isolates "TRS-1, TRS-2, TRS-3, TRS-4, TRS-5, TRS-7, and TRS-8" each exhibited their own distinct Plant Growth-Promoting (PGP) activities. Only TRS-5 exhibited phosphate solubilization capabilities when tested. "TRS-1, TRS-3, and TRS-8" stood out among the seven examined isolates for their exceptional competence in phytolase production and zinc solubilization. Each individual test isolate exhibited the development of hydrogen cyanide (HCN), siderophore, and indoleacetic acid (IAA). However, there was no evidence of chitin hydrolysis in any of the samples used. Isolates "TRS-1" and "TRS-7" were determined to have biofilm, whereas "TRS-2, TRS-4, TRS-7, and TRS-8" were shown to have detectable ACC deaminase production. Research was conducted to assess the antagonistic activity of fungus and bacteria isolated from rhizobacterium, including species of Fusarium and Curvularia, as well as Xanthomonas oryzae pv. citri and Xanthomonas axonopodis pv. oryzae. There was no indication that any of the isolates had any activities that were hostile to Fusarium species. Although both "TRS-1 and TRS-5" demonstrated antibacterial activity against Xanthomonads (Xoo and Xac), the ability to restrict the growth of Curvularia species was only shown by TRS-1.

Table 2: Assessment of plant growth-promoting activities in rhizobacterial isolates (Kalam et al., 2020)

Isolates	CP	PS	ZS	SP	IP	HP	BF	PP	AD	Antibacterial		Antifungal	
										Xoo	Xac	F	C
TRS-1	-	-	++	+++		-		+++	+	-	+	+++	-
TRS-2	-	++	+	+++	+	+	+++	+++	+	-	++	++	-
TRS-3	-	+++		-	+++	+		+++	+	+++	-	-	-
TRS-4	-	+++	+	-	+++	+++	-	-	+	+++	-	-	-
TRS-5	+++	+	++	-	+++	+	-	-	+	-	-	-	-
TRS-7	-	-	+	+	+++	+	-	++	+	-	+	+	-
TRS-8	-	++	+	+	-	-	-	+	+	++	+++	+	-

Note:

"PS: Phosphate Solublisation

CP: Chitinase production

SP: Siderophore production

ZS: Zinc Solublisation

HP: HCN production

IP: IAA production

PP: Phytase production

BF: Biofilm formation

AD: ACC deaminase activity"

For the purpose of phosphate and zinc solubilization, the generation of siderophores and chitinases, as well as antibacterial and antifungal laboratory tests:

- '"**+**': Positive result, zone of clearance < 0.2 mm
- '**++**': Positive result, zone of clearance 0.2–0.4 mm
- '**+++**': Positive result, zone of clearance > 0.4 mm"

→ **For IAA production:**

- '"**+**': Positive result, absorbance < 0.1
- '**++**': Positive result, absorbance 0.1–0.3
- '**+++**': Positive result, absorbance > 0.3"

→ **Test Microorganisms:**

- "Xac: *Xanthomonas axonopodis* pv. citri
- Xoo: *Xanthomonas oryzae* pv. oryzae
- C: *Curvularia* sp.
- F: *Fusarium* sp."
- **Gene-based molecular characterization of 16S rRNA:** The length of the amplicons produced during the Amplification of the 16S rDNA via PCR was determined to be 1500 base pairs. A NCBI-BLAST analysis was performed on sequences of the 16S rRNA genes in their entirety present in the rhizobacterial isolates that were tested. Upon analysis, it was determined that all seven isolates are classified under the *Bacillus* genus, as evidenced by their 99-100% similarity to other *Bacillus* members (refer to Table 3).

Table 3: The categorization of rhizobacterial isolates is established based on the similarity of their 16S rRNA gene sequences (Kalam et al., 2020)

Isolates	Medium of Isolation	NCBI Strain	Percentage of Similarity	Accession Number
TRS-7	M2	*Bacillus sp.*	98	KJ572790
TRS-2	M1	*Bacillus subtilis*	100	KJ572791
TRS-1	M1	*Bacillus licheniformis*	98	KJ572792
TRS-4	M2	*Bacillus sp.*	98	KJ572792
TRS-3	M1	*Bacillus pumilus*	99	KJ572793
TRS-5	M2	*Bacillus sp.*	100	KJ572794
TRS-8	M2	*Bacillus sp.*	98	KJ572795
In order to determine the phylogenetic and homology of the rhizobacterial isolates, their 16S rRNA gene sequences were compared to those of comparable strains in the NCBI database.				

- **BOX-PCR analysis:** An optimization of the settings for BOX-PCR amplification was performed in order to generate fingerprint patterns that are both distinctive and informative (see Figure 1). The use of BOX-PCR analysis made it possible to differentiate between unique strains, which ultimately resulted in the development of Six unique fingerprinting profiles or electrophoretic patterns were identified for each of the seven isolates under investigation. Particularly noteworthy is the fact that isolates "TRS-2 and TRS-4" had the same patterns. It was discovered via the profiles that there were a considerable number of polymorphic bands with varying intensities and sizes that ranged from 0.2 to 3.0 kilobases. In order to determine the consistency of these patterns and to ensure that they are stable and repeatable, the experiment was carried out in triplicate (n = 3), hence guaranteeing that they are consistent. Every single one of the isolates had a banding pattern that is characteristic of the *Bacillus* genus. Nevertheless, in a number of the bands, the intensities of the bands from isolates "TRS-1, TRS-2, TRS-3, and TRS-4" were notably high.

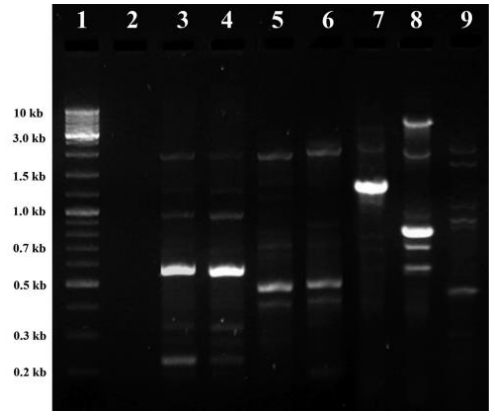

Figure 1: BOX-PCR patterns of the seven Bacillus genus isolates labeled as TRS (Kalam et al., 2020)

The visual depiction illustrates a sequence of entities arranged in a sequential fashion from left to right. A Generationer 2-Log DNA Ladder from New England Biolabs in the United States of America was the supplier of the DNA molecular mass standard that is located in Lane 1. The left-hand margin has a list of the measurements that are associated with Lane 1. Lane 2 is comprised of sterile water that is free of any DNA and acts as the control for the experiment. *Bacillus* species "TRS-2, TRS-4, TRS-7, TRS-8, TRS-1, TRS-3, and TRS-5" are represented by the lanes 3–9 in this diagram.

- **Phylogenetic tree:** A phylogenetic tree was generated by utilizing the sequences of the 16S rRNA gene; this tree demonstrated that there is a significant amount of genetic similarity between the seven Bacillus isolates (see to Figure 2 for further information). Four distinct groups were formed using isolates: the first, second, third, and fourth groups, respectively, contained two, two, one, and two isolates. These groups were produced from the various isolates.

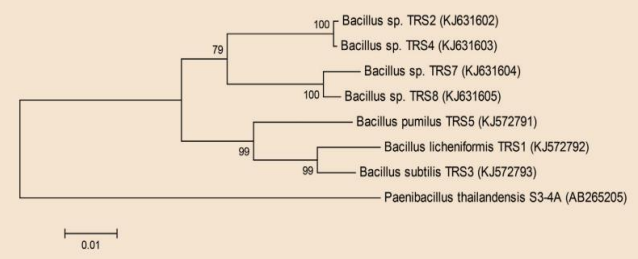

Figure 2: Phylogenetic tree utilizing 16S rRNA gene sequences to illustrate the relationships between the seven TRS isolates (Kalam et al., 2020)

Using the sequences of the 16S rRNA gene, a phylogenetic tree was created in order to illustrate the genetic connections that exist between the seven TRS isolates. A Bootstrap value of one thousand was applied to a Neighbor-Joining tree-based evolutionary analysis in order to ascertain the genetic relatedness. For the purpose of this experiment, the outgroup was *Paenibacillus thailandensis* S3-4A, which has the accession number AB265205 in GenBank. The MEGA6 program was utilized in the phylogenetic tree's construction. The proportion of replicated trees that had clusters of the respective taxa is represented by the numerical values that are assigned to the nodes in the tree. With the scale bar denoting a rate of 0.01 substitutions per nucleotide location, the tree is presented in an accurate manner.

- **Plant growth promotion in tissue culture:** Figure 3 illustrates how the development of tomato seedlings was evaluated in response to Bacillus isolates by subjecting the seeds to treatment with the isolates and subsequently observing their reaction in an MS medium. Figure 3A illustrates alterations in the root length of tomato plants that were exposed to rhizobacteria. Comparing the seed bacterization induced by TRS-4 and TRS-5 to that of the control groups revealed no significant differences. However, by contrast with the CMC control, the length of the tomato roots increased significantly in all five remaining treatments, including the commercial isolate (CBli). Notably, in comparison to the other isolates, TRS-8 demonstrated the most substantial growth in root length. (Figure 3B) There was substantial variation in the branch length response to bacterial isolates. When compared to the other isolates, the "TRS-2, TRS-4, TRS-7, and TRS-8" isolates exhibited a larger degree of branch length augmentation than the other isolates. The limb length of plants derived from seedlings infected with "TRS-8, TRS-7, TRS-2, and TRS-4" increased significantly in comparison to the control (CMC) and CBli.

As a result of seed bacterization, there was considerable variation in the increase in dried weight, specifically with "TRS-2, TRS-7, and TRS-8" (Figure 3C). The application of TRS-8, which was then followed by TRS-7, as a result of which there was a substantial rise in the dry weight of the plants in comparison to the group that was treated with CMC as the control. Additionally, the rhizobacterial isolates were examined to determine whether or not they were able to colonize the roots (Figure 4). The colonies that were formed on tomato roots were successful for all of the isolates. Following a period of five days, it was discovered that the root colonization of isolates TRS-1 and TRS-3 had significantly increased. All of the isolates, with the exception of TRS-4, demonstrated considerable root colonization at both 10 and 15 different days. When compared to CBli, "TRS-8 and TRS-7" performed much better in terms of root colonization.

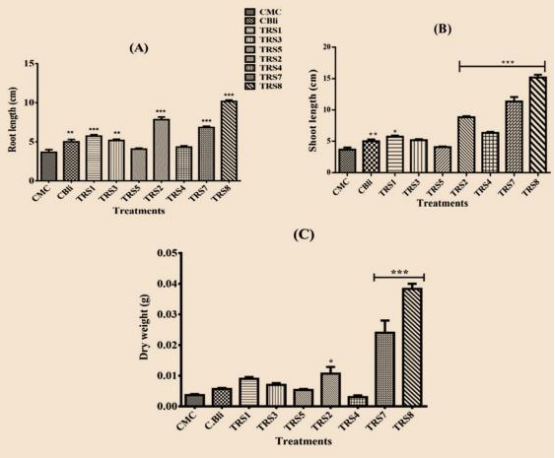

Figure 3: Seven rhizobacterial isolates, one commercial Bacillus licheniformis strain (CBli), and a control (CMC) were tested on tomato plants grown on MS medium for 15 days. Root length (A), stem length (B), and dry weight (C) were measured (Kalam et al., 2020)

The data that are shown are representative of the mean (n = 3), and the vertical lines represent the standard deviation that was used in the statistical analysis of the mean. At the right levels for each growth response, a statistical analysis was carried out. This study included the length of the shoots, the length of the roots, and the weight of the dried plant. The inquiry consisted of comparing several means using a one-way analysis of variance (ANOVA), which was then followed by Dunnett's post-hoc test. The significance levels are shown as *** for very significant, ** for moderately significant, and * for less significant in comparison to the CMC control ($p < 0.05$).

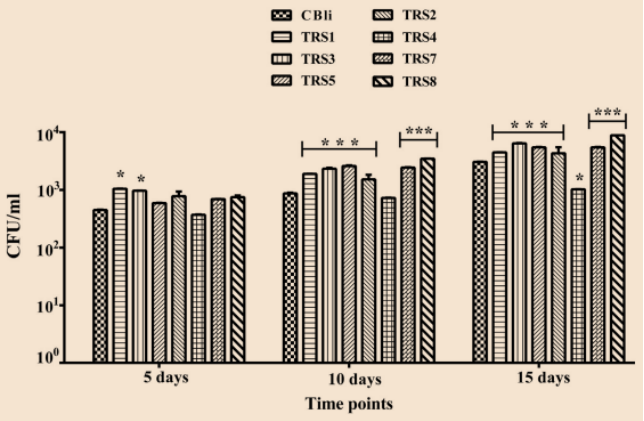

Figure 4: Evaluation of root colonization by the tested rhizobacterial isolates (Kalam et al., 2020)

The supplied numbers (n = 3) correspond to the average, and the vertical lines show the standard deviation of the mean. Meanwhile, the average is represented by the vertical lines. As a component of the statistical investigation, a two-way analysis of variance (ANOVA) was carried out on each of the test bacterial isolates at three different time periods. A Bonferroni post-hoc test was subsequently employed to compare the relative means of multiple groups. Significance levels are shown by three asterisks (***), indicating high significance, two asterisks (**), indicating moderate significance, and one asterisk (*), indicating low significance, as compared to the CBIi positive control ($p < 0.05$).

- **Promotion of plant growth in a conservatory:** The physicochemical characteristics of the greenhouse soil used in this study were as follows: it was red in color, sandy in texture, pH 7.5, oxidizable organic carbon in the range of 1% to 1.5%, ammoniacal nitrogen and nitrate nitrogen contents in the range of 10 to 15 kg/ha and 10 to 20 kg/ha, respectively, phosphorus availability in the range of 56 to 73 kg/ha, and potassium in the range of 112 to 280 kg/ha. Tomato plants that were subjected to bacterial treatment saw a gradual increase in the length of their shoots and roots, as well as an increase in their collective weight, according to the findings of an investigation that was carried out twenty, forty, and sixty days after the inoculation (refer to Figure 5). The investigation was carried out based on the findings of the investigation that was carried out. It was shown that isolating TRS-8 and TRS-7 had a significant influence on the growth parameters of tomato plants when compared to the CMC control at each of the time intervals that were provided. No matter how many different time periods were considered, this was always the case.

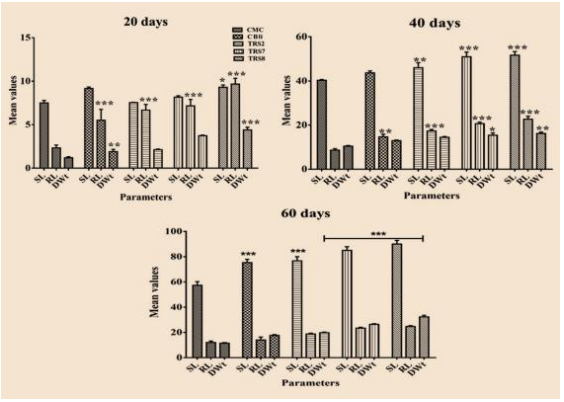

Figure 5: The effects of supplementing a commercial strain (CBIi) with three selected rhizobacterial isolates on the growth and development of tomato plants in a greenhouse over periods of twenty, forty, and sixty days: evaluation of dried weight, root length (RL in cm), and stem length (SL in cm). (Kalam et al., 2020)

It should be brought to your attention that all of the data that has been presented is representative of the average (n = 3), and the vertical lines represent the standard error of the average. A statistical investigation was carried out by using a two-way analysis of variance (ANOVA) for each growth response. This analysis included root length, shoot length, and dry weight as the variables of interest. Following this, a comparison of the means of many groups was conducted using the Bonferroni post-hoc test. Degrees of significance are shown as follows as compared to the CMC control: *** denotes a very significant result, ** a moderately significant result, and * a less significant result ($p < 0.05$).

CONCLUSION

A comprehensive analysis of *Bacillus* Plant Growth-Promoting Rhizobacteria (PGPR) isolated from the rhizosphere of tomato plants has provided valuable insights into their diverse and auspicious capabilities. The seven genotypes, denoted as TRS-1, TRS-2, TRS-3, TRS-4, TRS-5, TRS-7, and TRS-8, exhibited noteworthy attributes that facilitate the development of plants. The aforementioned characteristics consist of the capacity to phosphate and zinc solubilization, phytase, siderophore, and indoleacetic acid (IAA) production, biofilm formation, and ACC deaminase activity. In addition, the antagonistic activity of these strains against phytopathogenic bacteria and fungi underscores their potential for managing plant health. The confirmation of the isolates' classification as *Bacillus* members was achieved through genetic characterization employing 16S rRNA gene sequencing. Furthermore, the isolates' genetic diversity was demonstrated through the utilization of BOX-PCR fingerprinting. The strains effectively established colonies on the roots of tomato plants, thus showcasing their ability to develop mutually beneficial associations with their hosts. Experiments conducted in greenhouse conditions provided further evidence for the advantageous impact that specific

Bacillus isolates had on crucial tomato growth parameters, such as root length, stem length, and dried weight. The statistical analyses validated the significance of the noted enhancements, thereby furnishing substantiation for the pragmatic applicability of these PGPR strains in the context of sustainable agriculture. In general, the *Bacillus* PGPR strains procured from the rhizosphere of tomatoes exhibit potential not only for augmenting plant growth but also for biofertilization and methods of preserving plant vitality. The findings contribute to the growing body of knowledge regarding the utilization of beneficial rhizobacteria in sustainable agricultural methods, emphasizing the necessity for additional research into their specific functions and mechanisms.

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