

Growth dynamics and morphological changes in *Solanum lycopersicum* L. induced by rhizobacterial inoculation

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ABSTRACT

Numerous microbial populations exist in soil, which contribute to the rhizosphere community's variety through differences in genotype, phenotype and functional traits. These organisms fall under the category of plant growth-promoting rhizobacteria (PGPR), which is a collection of bacteria found in the rhizosphere that supports normal plant growth in the presence of biotic and abiotic stressors by the synthesis of phytohormones, increased enzymatic activity, induction of different PR-proteins, increased nutrients absorption and various other growth promotion processes. In this study, thirty distinct bacteria were isolated from the root and surrounding soil of *Solanum lycopersicum* L. plants, which were further categorized based on strong fungal antagonistic and distinguished plant growth promotion characteristics. Among these four isolates were selected based on remarkable study outcomes and the 2 best isolates were selected for molecular identification by the Sanger sequencing method. These isolates were identified as *Bacillus subtilis* and *Erwinia tasmaniensis* and employed as plant growth-promoting bacteria to support the tomato plant's better growth and high yield.

Key words: *Bacillus subtilis*, Biocontrol agent, *Erwinia tasmaniensis*, identification, PGPR, *Solanum lycopersicum*, tomato, 16SrRNA

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) rank second in terms of vegetable consumption (11). More than eighty percent of tomatoes produced worldwide are grown on the continents of Asia and Africa and is a significant crop in many countries (2). Tomato is a model plant to investigate various disease resistance mechanisms about genetic and molecular features and fruit development (3). Tomato is one of the most consumed vegetable in several countries like China, India, North Africa, the Middle East, USA and Brazil and its increasing demand is challenging to meet the requirement due to continuous limited availability of land and water resources. The growing demand for tomatoes is augmented by their high quality and quantity demand for households as well as for different industries.

Biotic stress on plants primarily results from various living organisms, including viruses, bacteria, fungi, nematodes, insects, arachnids and weeds. These stress agents damage plants by depriving them of nutrients, which weakens the plant and can even lead to its death in severe cases. Biotic stress negatively impacts crop quality and yield, alters plant physiology, reduces biomass, lowers seed production and triggers the accumulation of protective compounds. The Food and Agriculture Organization (FAO) estimates that biotic stress causes annual crop losses of 20 % to 40 % worldwide. According to Cochard *et al.* (4). tomato is host to around 200 plant pathogens, including bacteria, viruses, nematodes and fungi. This is one of the main reasons why tomatoes are subjected to increased stress conditions. Abiotic and biotic stressors caused a notable global decline in

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the tomato's overall yield and productivity. Tomatoes are susceptible to biotic stress conditions, as they are the host of several different plant pathogens. One of the most pervasive and ubiquitous fungal pathogens is *Fusarium oxysporumlycopersicum*, which causes massive damage to the tomatoes. *Fusarium oxysporum* has been known to cause an average of 80 % loss in tomato production (5).

The "plant growth promoting rhizobacteria (PGPR)," are beneficiary bacteria living in the plant's roots or the rhizosphere soil, which is close to the roots of the host plant (6). The tomato plant's rhizosphere is home to a diverse array of plant-growth-promoting rhizobacteria. PGPR can colonize plant roots, significantly increase soil fertility, promote plant growth and enhance crop yield. Furthermore, these PGPRs have the potential to benefit their host plants by promoting a variety of direct and indirect responses to overcome the effects of biotic stress and, as a result, enable plants to survive these stressful conditions. Availability of plant nutrients is crucial for crop production and PGPR is the group of organisms that help the soil retain its nutrients and fertility. The fundamental to sustainable agriculture is to ensure a steady supply of high-quality food for the growing global population. At present to retain tomato productivity chemical fertilizers and pesticides are used in an uncontrolled manner, responsible for the adverse effect of chemicals on the ecosystem as well as incorporation in the food chain. In recent years, there has been a growing interest in using such beneficial bacteria as an eco-friendly alternative to chemical fertilizers, which can degrade soil health and contribute to pollution. These microorganisms help plants to cope with both biotic and abiotic stresses, while also enhancing growth and yield in an eco-friendly manner (7). The present study was focused on the isolation, characterization and identification of PGPR from tomato plant rhizosphere and its positive effect on plant growth.

MATERIALS AND METHODS

Isolation and characterization of PGPR from *Solanum lycopersicum* plants: This study was carried out at Anand district of Gujarat, India (22.3043 °N, 73.4017 °E), situated at an elevation of approximately 54 m above sea level and experiences a tropical climate. The average annual temperature is 27.2 °C (81.0 °F) with average annual rainfall of 869 mm. The soil associated with the roots was tapped in 100 ml of sterile distilled water and allowed to settle down for 10 min. The supernatant of the soil sample was used for the isolation of rhizosphere bacteria. While for the isolation of endophytic bacteria, the roots were surface sterilized and crushed in sterilized distilled water. The root suspension (10⁻⁶ dilution) was inoculated into nitrogen-free Ashby's mannitol agar solid medium and Burks N-free semi-solid media and tubes were incubated for 24 h at 30 °C.

All the isolates were analyzed for their plant growth promoting properties such as nitrogen fixation, fungal antagonistic activity, mineral solubilization (potassium, phosphate, zinc), ammonia production, IAA production, HCN production and enzymatic activity. Along this the study of colonial characteristics, gram staining and CFU/ml of all cultures were also carried out.

Analysis of fungal antagonist activity (biocontrol activity) of PGPR: All the 30 isolates were tested for their antagonistic ability against the pathogenic fungus *Fusarium oxysporum* on the sterilized PDA solid media by plating the two microorganisms side by side in a plate. In this one half was streaked with bacterial culture and the second half with

a disc of fungus culture. All the plates were incubated at 30 °C for 7 days (8). The percent inhibition was calculated using the formula:

$$PI = (R-r) / R \times 100$$

Where, PI : Inhibition (%)

R : Radius of fungal growth in control plate.

r : radius of fungal colony opposite the bacterial colony.

Analysis of nitrogen fixation ability of isolated PGPR: Among 30 different isolates, 12 isolates based on their antagonistic ability were selected for assessing their nitrogen fixing ability on N-free media. All 12 isolates were inoculated in Burks liquid N-free media and incubated for 24 h at 30 °C (2,3). After the incubation 0.5 ml of bromothymol blue dye was added in all 12 flasks containing bacterial cultures to observe the color change due to change in pH.

Analysis of mineral solubilization by PGPR: Twelve PGPR isolates based on their ability to fix atmospheric nitrogen, antagonistic potential against fungus *Fusarium oxysporum* and mineral solubilization activity were selected.

(i). Phosphate Solubilisation: Inorganic phosphate solubilisation by the isolates was determined on Pikovskaya's agar medium[9], by incubating the inoculated plate for 3 days at 28 °C.

(ii). Potassium solubilisation: Inorganic potassium solubilization by the isolates was determined on sterilized Aleksandrov agar media, by incubating the inoculated plate for 48 h at 28 °C.

(iii). Zinc solubilization: The zinc solubilising the bacteria was tested on sterilized zinc agar media and incubated for 7 days at 30°C temperature.

The Mineral solubilization index for all 3 minerals was calculated by using formula-
 SI (solubilization index) = colony diameter + zone of clearance/colony diameter (cm).

Analysis of ammonia production by PGPR: Bacterial isolates were tested for ammonia production in peptone water as per Devi *et al.* (5). Freshly grown cultures were inoculated in 10 ml of peptone water and incubated for (48-72 h) at 30 °C. After incubation 0.5ml of Nessler's reagent was added to observe color change in media.

Analysis of Indole Acetic Acid Production by PGPR: For screening for the ability to produce indole acetic acid, all the test cultures were inoculated in LB broth amended with tryptophan and incubated for 7 days at 28°-30 °C temperature. After that the tubes were centrifuged at 3000 rpm for 30 min and 2 ml of supernatant was mixed with a few drops of ortho-phosphoric acid and 4 ml of Solawaskis reagent for color change (10).

Analysis of HCN Production by PGPR: To analyze the capability for HCN production, the bacteria were inoculated in nutrient agar medium was amended with glycine 4.4 gL⁻¹. A Whatman filter paper no 1 was soaked in a solution containing 2 % sodium carbonate + 0.5 % picric acid and placed under the lid of the Petri plate (5). The plates were then sealed with parafilm and incubated at 30 °C for 4 days. After incubation the plates were observed for color change in the filter paper.

Estimation of enzymatic activity of the isolated PGPR:

(i). Catalase assay: The catalase activity of the bacterial isolates was carried out by Fatima *et al.* (8) and Geetha *et al.* (9) method. In this method 24 h old cultures were spotted on a grease free glass slide and 3-4 drops of hydrogen peroxide were added to the culture spots at the slide for appearance of effervescence.

(ii). Amylase assay: Amylase activity of the selected isolates was checked by streaking it on sterilized starch agar media plates and incubated at 30°C for 48 h. After incubation all the plates were flooded with iodine solution, a clear zone around the bacterial colonies indicated a positive amylase test.

(iii). Caseinase assay: The Casienase/protease activity of the selected isolates was analyzed on a sterile skim milk agar plate (1 % skim milk was added to Nutrient agar). Spot inoculation technique was used on Skim milk agar plates and then the plate was incubated for 48 h at 30 °C for the formation of a clear halo zone around the bacterial colony.

Molecular identification of bacterial isolates: The colony PCR was used for the molecular identification of isolates using the universal primers 16S-1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') and 16S-27F (5'-CCA GAG TTT GAT CMT GGC TCA G-3') with standard PCR reaction condition. The obtained PCR product was analysed on Agarose gel. The DNA from the bands were eluted and after purification, the amplified PCR product was cycle-sequenced using BDT v3.1 chemistry and sequenced on an ABI 3500XL Genetic Analyzer.

After sequencing, the trace files were saved as a FASTA read file, which was then manually edited, and the consensus sequence was searched in a database using the BLAST tool (1). Among the top 1000 matches with the highest similarity in the search results were taken from the database and aligned using the MUSCLE aligner for the phylogenetic analysis (6). A consensus phylogram was created using the neighbour joining algorithm with maximum likelihood and phylogenetic tree was constructed.

Plant Inoculation with PGPR and Experimental Setup: The experiment commenced on January 1, 2025 and concluded on January 31, 2025. The experimental design consisted of different bacterial inoculum treatments to assess their effects on plant growth. PGPR was applied using direct soil drenching or root inoculation methods to maximize root colonization and enhance plant-microbe interactions. The bacterial culture (10 ml) were applied every 7 days to maintain a consistent cell count throughout the experiment. Total five pots were used, each representing a distinct inoculum sample, with two replicates per treatment. One set of replicates received only the PGPR inoculum, while the other set was treated with both PGPR inoculum and *Fusarium* infection. This design allowed for a comparative analysis of plant responses to PGPR treatment alone and in combination with fungal infection. The following stated are the experimentally treated PGPR inoculum in each pot.

Sample 1 (Control): No bacterial inoculum (untreated control).

Sample 2: *Erwinia tasmaniensis* (E.T) inoculation.

Sample 3: *Bacillus subtilis* (B.S) inoculation.

Sample 4: Combination of *E. tasmaniensis* and *B. subtilis*.

Sample 5: Consortium of *E. tasmaniensis*, *B. subtilis*, AN2308, AN2509 and AN2910.

Effects of selected PGPR on the Morphology of Plants: The following morphological studies were done by uprooting the plants, at regular intervals (7, 14, 21 and 28 days) to analyse plant development:

Seed Germination Rate: % of seeds that successfully sprouted.

Root Length : Length of the primary root system.

Shoot Height : Growth of the shoot from the base to the tip.

Plant Height : Total height from root base to shoot tip.

Number of Leaves : Count of fully developed leaves.

Fresh and Dry Weight: Fresh and dry biomass weight to assess growth.

Chlorophyll Content : Indirect indicator of plant health and photosynthetic efficiency.

RESULTS

Isolation of PGPR and analysis of antagonist activity: A total of thirty pure cultures were isolated from the root samples of the tomato plant, using spread plate technique on solid media. Among the thirty isolated bacteria twelve isolate showed strong antagonism against fungal pathogen *Fusarium oxysporum* and potential of its % inhibition activity as shown in Table-1.

Table 1. The Antagonistic % inhibition and Mineral solubilization indexes for phosphate, potassium and zinc of the selected isolates (psi = phosphate solubilization index; ksi = potassium solubilization index; zsi = zinc solubilization index).

Sample	CFU/ml	Percent inhibition (%)	Psi (phosphate solubilization index)	Ksi (potassium solubilization index)	Zsi (zinc solubilization index)
1	-	71.87	6	4.09	0
2	1.5×10 ⁹	71.25	3.9	3.42	0
3	2.1×10 ⁹	68.12	0	0	0
4	1.8×10 ⁹	70	2.6	3.9	0
5	1.2×10 ⁹	70	3.5	3.9	0
6	1.5×10 ⁹	70.62	5.2	0	0
7	6.8×10 ⁸	68.12	0	0	3.25
8	-	63.75	0	0	2.6
9	1.01×10 ⁹	68.12	6	3.7	2.3
10	1.2×10 ⁹	68.62	0	3.2	4.3
11	1.3×10 ⁹	66.25	0	0	0
12	1.6×10 ⁹	67.5	4.33	0	0

Analysis of nitrogen fixation ability and cultural characteristics of isolated PGPR: All twelve selected isolates showed some growth in Burks liquid N-free medium and the addition of pH indicator dye bromothymol blue changed from green to blue indicating positive nitrogen fixation. However, nitrogen fixation ability is subject to confirmation by other specific tests.

Mineral solubilisation (phosphate, Potassium, zinc): The formation of clear zones on mineral specific media has been clearly observed on different selective media for minerals like potassium, phosphorus and zinc, which indicate the mineral utilization by bacterial isolate as shown in Table-2.

Ammonia production: All the twelve culture tubes after addition of 0.5ml of Nessler's reagent showed different range of brown to yellow colour change indicated positive ammonia producer shown in Table-2.

Indole acetic Acid production: All the 12- culture suspension obtained after 48 h of incubation and centrifugation in presence of 4 ml of Solawaskis reagent with a few drops of orthophosphoric acid showed colour change from pink to orange indicating successful IAA production by the isolates (Table-2).

Table 2: The comparative analysis of Plant growth characteristics for selection of potent isolates.

Culture No.	API	psi	ksi	zsi	HCN	NH ₃	IAA	Amylase	Catalase	Caseinase
1	72	6	4.09	0	+	++	+++	++++	+++++	+++++
2	71.25	3.9	3.42	+	0	++	+++	0	+++++	+++++
3	68.12	0	0	0	0	++	+++	++++	+++++	0
4	70	2.6	3.9	0	0	++	+++	++++	+++++	0
5	70	3.5	3.9	0	+	++	+++	0	+++++	+++++
6	70.62	5.2	0	0	+	++	+++	++++	+++++	+++++
7	68.12	0	0	3.25	0	++	+++	++++	+++++	+++++
8	63.75	0	0	2.6	0	++	+++	++++	+++++	0
9	68.12	6	3.7	2.3	0	++	+++	0	+++++	0
10	68.62	0	3.2	4.3	+	++	+++	++++	0	0
11	66.25	0	0	0	0	++	+++	0	+++++	0
12	67.8	4.33	0	0	+	++	+++	++++	+++++	0

(API= antagonistic percent inhibition, psi= phosphate solubilization index, ksi= potassium solubilization index, zsi= zinc solubilization index, HCN= hydrogen cyanide, NH₃= ammonia, IAA= indole acetic acid).

HCN production: In this study, all of the twelve selected isolates produced HCN, at different levels observed as the pink/orange color change of Whatman filter after incubation (Fig.1; Table-2).

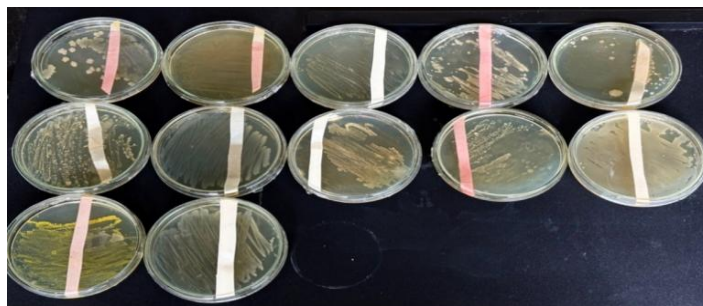


Figure 1. Characterization of bacterial HCN (hydrogen cyanide) production by selected isolate.

Catalase activity: Twenty four-h old bacterial culture from all twelve selected isolates was spotted on grease-free glass slide in presence of H_2O_2 , instant effervescence (Fig. 2), indicated the catalase producing potential of the isolates (Table-2).

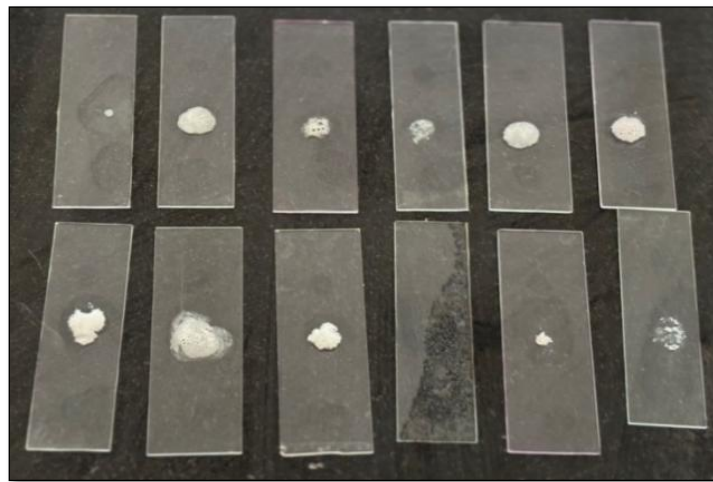


Figure 2. Characterization of catalase activity of the selected isolates.

Amylase and Caseinase activity of selected PGPR: The culture plates of all of the twelve selected isolates were flooded with iodine develop the blue colour (Fig. 3), which faded immediately and a clear zone around the bacterial colony depicted positive Caseinase activity (Fig. 4, Table-2).

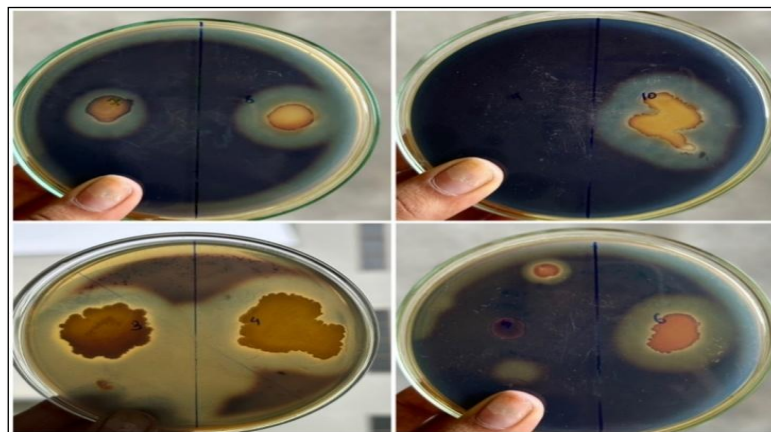


Figure 3. Characterization of amylase Activity of the selected isolates.

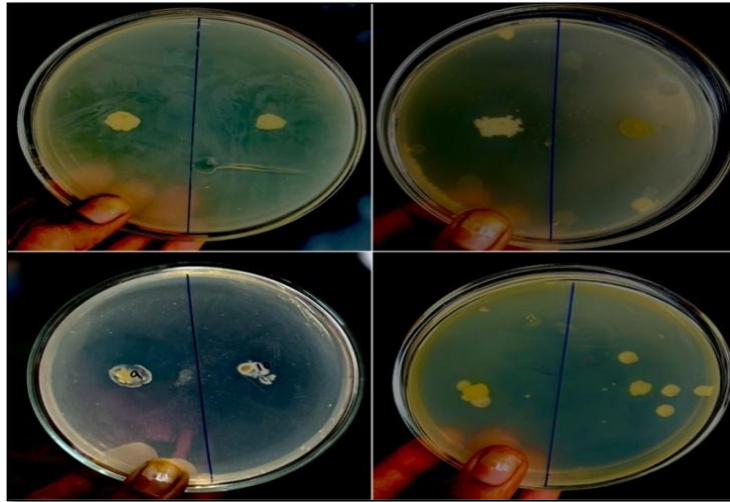


Figure 4. Characterization of caseinase activity of the selected isolates.

Molecular identification: The phylogenetic trees were constructed using BLAST software by the comparison of the 16S rDNA sequence of isolates and related genera from a database using the neighbor-joining (NJ) algorithm and maximum likelihood (ML) method, which are shown in Figure 5 and 6 respectively. Nucleotides homology and phylogenetic analysis of the selected isolate was identified as *Erwinia tasmaniensis* and *Bacillus subtilis*.

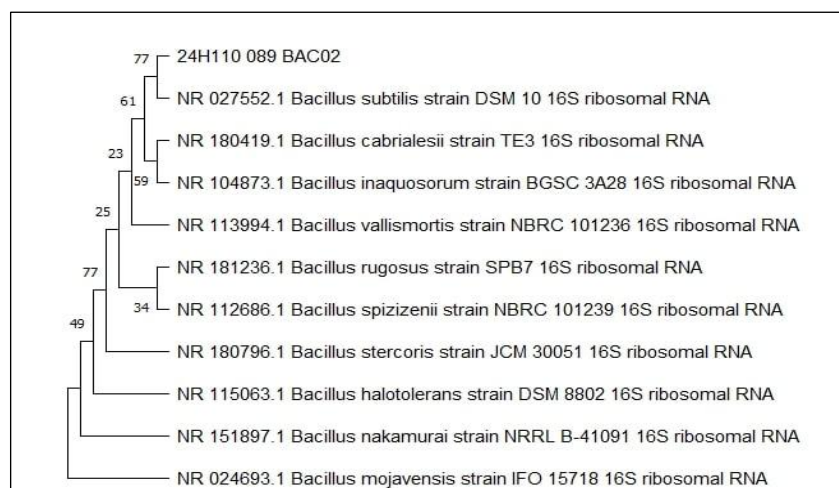


Figure 5. Phylogenetic tree data of *Bacillus subtilis*



Figure 6. Phylogenetic tree data of *Erwinia tasmaniensis*.

Plant growth Assessment of PGPR-Inoculated tomato plants: To check the effect of selected bacteria on tomato plant inoculated with isolates showed consortium had the most pronounced enhancement in plant morphology, surpassing the effects observed in both single and dual inoculations. Plants treated with the bacterial consortium demonstrated superior root and shoot development, suggesting a synergistic effect of multiple bacterial strains in promoting growth. The study extended over 28 days and comparative analysis across this time revealed a consistent trend of superior growth performance by the consortium-treated plants, highlighting its potential as an effective bio-inoculant strategy for tomato cultivation. Additionally, comparative graphs highlight the effects of PGPR treatments providing insights into plant growth dynamics against stress tolerance (Fig.7 and 8).



Figure 7. Morphological Examination of Inoculums Treated Plants on Day 21 and Day 28

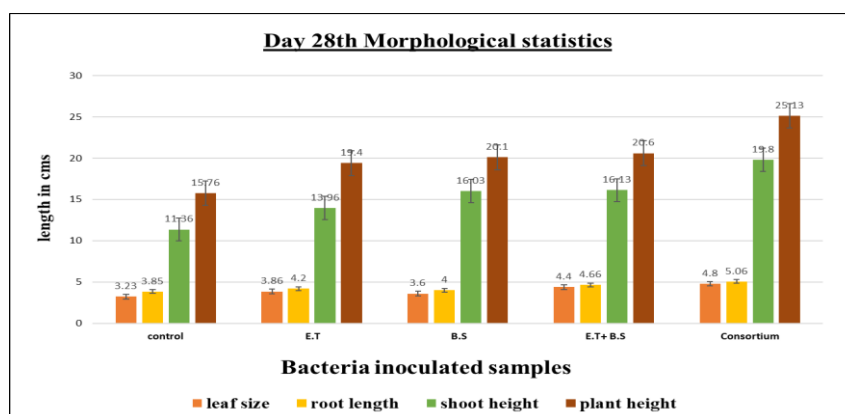


Figure 8. Graphical representation of growth parameters with different inoculums on day 28th

DISCUSSION

PGPR colonize plant roots and exert beneficial effect on plant growth and development by wide variety of mechanism. Rhizosphere is one of the most dynamic ecological niches where inter and intra species interactions of microbes, such as bacteria, fungi and protozoa, occur due to the presence of rich and diverse microbial food source. The rhizosphere microbial population helps in maintenance of root health by enhancing nutrient uptake and tolerance of environmental stress is well recognized. The rhizosphere bacteria are isolated from plant roots using several processes (11) and to isolate particular bacterial groups, different selective media is utilized.

PGPR can induce plant defence responses, enhancing the plant's resistance to fungal infections. This natural biocontrol reduces the need for chemical fungicides, supports healthier plants, and contributes to improved crop yield and quality. In this study, among thirty isolates, twelve isolates showed antagonistic effect against fungus, which can be due to production antifungal compounds like antibiotics and volatile organic compounds, competing for nutrients and space and secreting enzymes that degrade fungal cell walls. Such PGPR can act as natural biocontrol to reduces the need for chemical fungicides and contributes to improved crop yield and quality (15)

The nitrogen is a fundamental component of vital macromolecules like proteins, DNA, and chlorophyll, it is crucial for plant growth and agricultural productivity (12). Although nitrogen is abundant in the atmosphere, but plants are unable to directly use N_2 , thus nitrogen fixation is necessary to release nitrogen in a form that is useful to plants. In this study, all the twelve bacteria cultured on the nitrogen-free media for 24 h at 30 °C and addition of pH indicator dye showed change in colour, indicate all are nitrogen fixer. The nitrogen fixation helps to provide organic nitrogen to the soil, enhancing its general fertility and structure, lowering the need for synthetic nitrogen fertilizers and assisting in maintaining a consistent supply of nitrogen for enhanced crop yields (13).

To improve plant nutrition and encourage sustainable farming practices, this bacterial activity is essential. The PGPR convert insoluble forms of minerals as phosphorus, potassium, and zinc into soluble forms that plants may absorb through their roots by producing organic acids like citric acid, gluconic acid and oxalic acid. Phosphorus is a vital nutrient for plants, playing a key role in energy transfer, photosynthesis and root development. It is an essential component of ATP and is also part of DNA, RNA and phospholipids, which are crucial for cellular processes. Phosphorus helps promote strong root systems, improves flowering and fruiting and enhances overall plant growth and resistance to stress. A deficiency in phosphorus can lead to stunted growth, poor root development, and reduced yields. Potassium is an essential nutrient for plants, playing a crucial role in regulating various physiological processes, including water uptake, enzyme activation and photosynthesis. It helps maintain proper cell turgor, which is important for plant structure and hydration and supports the synthesis of proteins and starches. Potassium also enhances the plant's resistance to stress, including drought, disease and extreme temperatures, while promoting strong root development and overall plant vigor. Similarly zinc is another essential micronutrient for plants, playing a critical role in various biochemical processes, including enzyme activation, protein synthesis and hormone regulation. It is involved in chlorophyll production, contributing to healthy leaf development and photosynthesis. Zinc also supports the formation of starches and enhances the plant's resistance to disease. In this study, out of selected 12 antagonist bacteria, allowed to grow on the specialized growth media such as Pikovskaya's agar, Aleksandrov's agar and zinc agar are utilized to select bacteria that solubilize minerals such as zinc, phosphorus and potassium. The phosphate-solubilizing bacteria produce clear halos around their colonies by secreting organic acids that dissolve insoluble phosphate on Pikovskaya's agar (9). In this study, out of selected 12 antagonist bacteria, 7 solubilize phosphate, 6 solubilize potassium and 5 solubilize zinc.

The HCN and ammonia released by the PGPR plays a signalling role in the interaction between PGPR and plants. The result in present study showed that 5 isolates were positive for HCN production. Hydrogen cyanide has a significant role in plant defence against pathogenic infections (2). Bacterial HCN also stimulates the growth of plants, promotes root development, increase nutrient availability and boost plant health. Ammonification is the process of transformation organic nitrogen into forms that are readily absorbed by plants, such as nitrate and ammonium (16). In this study all the isolates had the ability for the ammonia production, which makes sure that plants have a constant supply of nitrogen key component of sustainable agricultural methods, is the production of bacterial ammonia (16).

PGPR produce various plant hormones, such as auxins, cytokinin and gibberellins, which play key roles in promoting plant growth. Auxins stimulate root development and cell elongation, enhancing nutrient and water uptake. Plants' responses to environmental cues and a variety of physiological processes, such as cell elongation, division and differentiation, are influenced by auxins, especially IAA (16). Nutrient Broth with Tryptophan is a basic growth medium serves as a precursor for IAA synthesis and in this study, the synthesis of IAA was detected in all 12 selected isolates.

Amylase and protease produced by PGPR play important roles in enhancing plant growth. Amylase breaks down starches into simpler sugars, providing plants with easily accessible energy and promoting root and shoot development (19). Protease, on the other hand, breaks down proteins into amino acids, which are essential for plant growth and the synthesis of enzymes and other proteins. By producing these enzymes, PGPR help improve nutrient availability, promote better plant growth and enhance overall plant health, especially in nutrient-limited conditions. Catalase enzyme is important in preventing oxidative damage to cells by hydrolysing hydrogen peroxide (H_2O_2). Pathogen invasion also induce oxidative damage is reduced by catalase in the plant and it is essential for maintaining cellular redox balance and signalling pathways, crucial for plant growth and development. In this current study, out of 12 isolate number 10 isolates exhibited good catalase activity, 8 isolates were amylase producers and 5 isolates showed positive caseinase activity. Plant health and defense depend on enzymes for several biochemical processes necessary for growth, development, and environmental adaptation (18). On the basis of all the PGPR characterization assay, two isolates (AN01 and AN02) showing best attributes of the twelve were selected for molecular identification and were identified as *Erwinia tasmaniensis* and *Bacillus subtilis*. Till date there is no evidence of *E. tasmaniensis* having a PGPR effect on tomato plants, as indicated in the literature. However, this organism is known to function as an epiphytic antagonistic bacterium that fights the gram-negative bacterium *Erwinia amylovora*, which causes fire blight in apples and pears. *Bacillus subtilis* on the root surface has been reported to defend against *Fusarium oxysporum*. Out of the twelve bacterial isolates another three promising strains (5/AN05, 6/AN06 and 7/AN07) were selected to formulate a microbial consortium with *Erwinia tasmaniensis* and *Bacillus subtilis*. To evaluate the impact of PGPR consortium on the morphological parameter such as leaf size, plant height, root length and shoot length of tomato plant were systematically recorded to assess the comparative effectiveness of these treatments in this study. Results demonstrated that plants inoculated with the bacterial consortium exhibited superior growth compared to those treated with single bacterial strains or dual mixed cultures. Consortium-treated plants showed significantly increased root and shoot elongation, enhanced leaf expansion and greater overall biomass accumulation. These improvements suggest a synergistic effect among the bacterial strains, leading to enhanced nutrient uptake, better root colonization and increased plant vigour. This finding indicates that the additional bacterial strains in the consortium played a crucial role in amplifying the beneficial effects, possibly through cooperative metabolic interactions.

The findings of this study highlight the potential of PGPR consortia as an effective biofertilizer strategy for improving tomato plant growth. The observed synergy between bacterial strains suggests that a well-optimized microbial consortium can out perform individual PGPR treatments by providing multifaceted benefits to plants. However, further research is required to validate these findings under field conditions and to elucidate the biochemical and molecular mechanisms underpinning the observed growth enhancement. Future studies should focus on optimizing bacterial strain combinations, assessing their long-term effects on crop yield and evaluating their role in mitigating biotic and abiotic stress factors. The application of PGPR consortia could contribute to sustainable

agricultural practices by reducing dependency on chemical fertilizers while enhancing crop productivity and soil health.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We did not exclude any author who substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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