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RESEARCH ARTICLE

Characterization of native root-knot nematode antagonistic rhizobacteria for plant growth promotion traits and their evaluation in tomato

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Abstract

In the present study, native root-knot nematode antagonistic rhizobacteria i.e., *Microbacterium laevaniformans*MlIRh3, *Staphylococcus kloosii*SkIRh4, and *Priestia aryabhattai* PaIRh7 from *Meloidogyne incognita* infected and *Staphylococcus sciuri* SsHRh3, *Bacillus pumilus* BpHRh5, *Priestia megaterium* PmHRh10 from uninfected tomato plants grown in the protected structure were characterized for their key plant growth promotion traits and survivability in soil, *viz.* phosphate solubilization activity, indole acetic acid production, ammonia production, and substrate utilization profiles. Among rhizobacteria, *S. sciuri* SsHRh3, *B. pumilus* BpHRh5, *P. megaterium* PmHRh10, *M. laevaniformans* MlIRh3, and *S. kloosii* SkIRh4 showed phosphate solubilisation activities on Pikovaskaya's media. In the presence of tryptophan, the rhizobacteria *P. megaterium* PmHRh10, *M. laevaniformans* MlIRh3, and *P. aryabhattai* PaIRh7 produced indole acetic acid. Similarly, *S. sciuri* SsHRh3, *B. pumilus* BpHRh5, *P. megaterium* PmHRh10, *S. kloosii* SkIRh4, and *P. aryabhattai* PaIRh7 produced ammonia under laboratory conditions. Among the 35 substrates studied, rhizobacteria had different substrate utilization profiles,*S. sciuri* SsHRh3 utilized most of the substrates (18), followed by *P. aryabhattai* PaIRh7, *S. kloosii*SkIRh4, *M. laevaniformans*MlIRh3, *B. pumilus* BpHRh5 and *P. megaterium* PmHRh10which were able to use 17, 16, 15, 14 and 13 substrates, respectively. Furthermore, the application of these rhizobacterial isolates either singly or in consortia revealed that a consortium of all three rhizobacterial isolates i.e., SsHRh3 + BpHRh5 + PmHRh10 from nematode uninfested rhizosphere shown highest plant growth promotion activity compared untreated control and singly application. In contrast, *S. kloosii*SkIRh4 either singly or in consortia, no significant effect on plant growth was observed under the pot experiment. Overall, the study found that root-knot nematode antagonistic rhizobacterial isolates were a promising candidate for plant growth promotion activity. Using them alone or in combination can be a safe alternative to synthetic chemical nematicides for suppression of root-knot nematode incidence in tomatoes grown under protected environments.

Keywords: Rhizobacteria, phosphate solubilization, indole acetic acid production, ammonia production, substrate utilization, root-knot nematode, tomato.

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Introduction

Root-knot nematodes (RKNs) are the major limiting factor for horticultural production. Among them, vegetables grown in both open-field and protected environments is highly prone to RKNs (Collange et al.,2011; Gowda et al., 2019). Root-knot nematodes proliferate 10 to 30 folds higher in a congenial protected environment than in open-field cultivation (Rao et al., 2015) and inflict significant yield losses in vegetable crops. Tomato (*Solanum lycopersicum* L.) ranks third among vegetable crops extensively grown under protected structures, is highly prone to RKNs and suffers from its infection in the entire crop season. The root-knot nematode (RKN) species, i.e., *Meloidogyne incognita* and *Meloidogyne javanica* are widely distributed in India and cause 23% of tomato yield loss with an estimated 6035.20 million rupees of monetary loss annually (Kumar et al., 2020). Despite, the synthetic chemical nematicides being the most

effective, their negative impact on human health and the environment is discouraged for their utilization. Using most of the available nematicides in the market is difficult for tomato growers as they are expensive and known to cause phytotoxicity and cause residue problems (Giannakou et al., 2019). Thus, biocontrol agents (BCA) are emerging as alternative chemical nematicides (Gao et al., 2016; Gowda et al., 2018). In this endeavor, earlier studies identified several nematode antagonists, which are categorized into egg-parasitic fungi, trapping fungi, toxin-producing fungi, and antagonistic rhizobacteria.

In recent years, rhizobacteria have become promising bioagents because of facilitate plant growth and productivity and are also concurrently able to resist infection of a broad range of plant pathogens, including plant parasitic nematodes (PPNs) (Mhatre et al., 2019; Gowda et al., 2018; Gowda et al., 2022)Rhizobacteria mediates plant growth promotion (PGP) activity by direct and indirect mechanisms. In direct mechanism, they colonize plant roots and supply growth factors such as nutrients or hormones to plants. Besides rhizobacteria, fixes atmospheric nitrogen, solubilizes phosphate, potassium, and zinc, produces siderophore and phytohormones such as indole acetic acid, cytokinin, and gibberellin, and also alleviates various stress by secreting ACC (1-aminocyclopropane-1-carboxylate) deaminase enzyme. Indirectly enhance plant growth by suppressing plant pathogens, including PPNs (Kumar et al., 2021; Gowda et al., 2022). Since many bacterial taxa are well known for their beneficial effects on vegetable production and growth, various rhizospheric bacteria such as *Azospirillum, Azotobacter, Arthrobacter, Alcaligenes, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas,* and *Serratia* are extensively used for increasing vegetable production (Kumar et al., 2021). In addition, the strains of *Staphylococcus* (Shahid et al., 2019) and *Microbacteriumlaevaniformans* (Malboobi et al., 2009) are also linked with plant growth promotion. Our previous study, isolated and characterized potential root-knot nematode antagonistic rhizobacterial isolates, i.e., *Microbacterium laevaniformans* MIIRh3, *Staphylococcus kloosii*SkIRh4, and *Priestia aryabhattai* PaIRh7 from *Meloidogyne incognita* infested rhizosphere and *Staphylococcus sciuri* SsHRh3, *Bacillus pumilus* BpHRh5, *Priestia megaterium* PmHRh10 from the uninfested rhizosphere of tomato plants grown in the protected structurethrough *in-vitro* and *in-vivo* assays (Gowda et al., 2023). Further, potential volatile organic compounds (VOCs), including nematicidal compounds, were characterized through gas chromatography-mass spectrometry (GC-MS) analysis (Gowda et al., 2023).

Nevertheless, to improve a plant's own survival capacity against PPNs and other ecological constraints without "trading off" yield potential, the identification of PGP traits from the potential rhizobacteria is crucial. Thus, the selection of suitable rhizobacteria or its consortium based on potential nematicidal activity along with associated plant growth promotion traits helps in greater suppression of PPN incidence in different agricultural and horticultural crops. Thus, in the present study, above mentioned root-knot nematode antagonistic rhizobacterial isolates were selected for characterizing their plant growth-promoting traits and survivability in soil, i.e., substrate utilization profile under *in-vitro* and their *in-vivo* evaluation on tomato.

Materials and Methods

Nematode Antagonistic Rhizobacterial Isolates

Pure culture of nematode antagonistic rhizobacterial isolates *Staphylococcus sciuri*SsHRh3, *Bacillus pumilus* BpHRh5, *Priestia megaterium* PmHRh10, *Microbacterium laevaniformans* MIIRh3, *Staphylococcus kloosii*SkIRh4, and *Priestia aryabhattai* PaIRh7were maintained at the Division of Nematology, ICAR-IARI, New Delhi was used for different experiments.

Plant Growth Promotion Traits Associated with Nematode Antagonistic Rhizobacterial Isolates

Phosphate solubilization

Pikovaskaya's agar media (containing agar 15 g, glucose 10 g, $Ca_3(PO_4)_2$ 5 g, yeast extract 0.5 g, $(NH_4)2SO_4$ 0.5 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, $MnSO_4$2H2O 0.1 mg, $FeSO_4$ 0.1 mg in 1000 mL double distilled water) was inoculated with rhizobacteria at the center of 90 mm Petri plate and incubated for 48 hours at 28 ± 2°C. Phosphate solubilization activity was identified on the basis of the formation of a clear zone around the colony of each strain (Malboobi et al., 2009).

Ammonia production

Freshly grown nematode antagonistic rhizobacterial strains were inoculated to a sterilized test tube containing 10 mL of peptone water and incubated for 48 hours at 28 ± 2°C. After incubation, 0.5 ml of Nessler's reagent was added to each test tube. These test tubes were observed for color change in the peptone water. Ammonia production from each rhizobacterial strain was identified based on the change in color from brown to yellow (Dunca et al., 2007).

Indole acetic acid (IAA) production

Production of IAA by each strain was determined by following Salkowski's method using Van Urk Salkowski's reagent. Each strain was cultured on yeast malt dextrose broth (YMD broth) with tryptophan (2 mg/mL) and incubated at 28 ± 2°C for 4 days. After incubation, YMD broth cultures were centrifuged at 1000 rpm for 5 minutes to obtain the supernatant. One ml aliquot of the supernatant was mixed with 2 mL of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$ solution) and incubated for 20 minutes under dark conditions. Indole acetic acid production was observed

by visualizing the development of a pink-red color (Islam et al.,2016)

Substrate utilization profile of nematode antagonistic rhizobacterial isolates

A substrate utilization study was performed with the ready-to-use strips having 35 different substrates (Hi-Carbohydrate Kit, Himedia Laboratories Pvt. Ltd., India). The total 35 substrates were distributed among three strips part A (contains 12 tests), part B (contains 12 tests), and part C (contains 11 tests). Each strip well was inoculated with 10 µL of 24-hour grown fresh broth culture of rhizobacterial strain and incubated at 28 ± 2°C for 24 hours. The strip wells were observed for color change after 24 hours of inoculation and incubation. The ability of each strain to utilize available substrate in strips was observed by the change in the color of the inoculated strips in comparison to the control.

Effect of nematode antagonistic rhizobacterial isolates on tomato plant growth under pot conditions

About 2 kg of sterilized soil was placed in plastic pots with a bottom diameter of 13.5 cm, a height of 20 cm, and a top diameter of 20 cm. In each pot, one healthy seedling was transplanted (25 days old). Five days after plant establishment, the tomatoes' rhizosphere was drenched with respective treatments. The details of treatments are as follows: T_1- Untreated control (40 ml /plant); T_2- *Microbacterium laevaniformans* (MlIRh3) @ 40 mL (3.58×10^9 CFU/mL)/plant; T_3- *Staphylococcus kloosii* (SkIRh4) @ 40 mL (3.70×10^9 CFU/mL)/ plant; T_4- *Priestia aryabhattai* (PaIRh7) @ 40 mL (3.66×10^9 CFU/mL)/plant; T_5- MlIRh3 + SkIRh4 @ 40 mL/ plant; T_6- MlIRh3 + PaIRh7 @ 40 mL/plant; T_7- SkIRh4 + PaIRh7 @ 40 mL/lant; T_8- MlIRh3 + SkIRh4 + PaIRh7@ 40 mL/plant; T_9- *Staphylococcus sciuri* (SsHRh3) @ 40 mL (3.54×10^9 CFU/ mL)/plant; T_{10}- *Bacillus pumilus*(BpHRh5) @ 40 mL (3.62×10^9 CFU/mL)/plant; T_{11}- *Priestia megaterium* (PmHRh10) @ 40 mL (3.68×10^9 CFU/mL)/plant; T_{12}- SsHRh3 + BpHRh5 @ 40 mL/ plant; T_{13}- SsHRh3 + PmHRh10 @ 40 mL/plant; T_{14}- BpHRh5 + PmHRh10 @ 40 mL/ plant; T_{15}- SsHRh3 + BpHRh5 + PmHRh10 @ 40 ml/ plant. A completely randomized design with five replicates was used to conduct the experiment under pot conditions in the protected structure environment. Further, good agronomic practices were followed to maintain plants in pot conditions. Then, ninety days after rhizobacteria inoculation, observations on plant growth promotion traits, including shoot length, root length, fresh weight of root and shoot, and fruit yield, were recorded.

Statistical analysis

Prior to analysis, numerical data from pot experiments were square-root transformed. Analysis of variance (ANOVA) using PROC GLM (SAS, 2011) was undertaken on the transformed data, and back-transformed data only were presented. Further, Tukey's significance test values at the 5% significance level were used to compare relevant means.

Results and Discussion

Plant Growth Promotion Traits Associated with Nematode Antagonistic Rhizobacterial Isolates

The nematode antagonistic rhizobacterial isolates showed varied plant growth promotion traits like phosphate solubilization, indole acetic acid production (IAA), and ammonia production activities (Table 1, Figures 1-3). Among nematode antagonistic rhizobacterial isolates, *S. sciuri* SsHRh3, *B. pumilus* BpHRh5,*P. megaterium* PmHRh10, *M. laevaniformans* MlIRh3, and*S. kloosii*SkIRh4 showed phosphate solubilization activities on Pikovaskaya's media and formed a clear transparent zone around the rhizobacterial colony. In the presence of tryptophan, the rhizobacterial strains *P. megaterium P. megaterium* PmHRh10, MlIRh3, and*P. aryabhattai* PaIRh7 produced IAA (Table 1). Similarly, *S. sciuri* SsHRh3, *B. pumilus* BpHRh5, *P. megaterium* PmHRh10, *S. kloosii*SkIRh4, and *P. aryabhattai* PaIRh7 produced ammonia under laboratory conditions (Table 1).

Rhizobacteria play a crucial role in nutrient assimilation, secreting and modulating hormones, secondary metabolites, and various signaling compounds, which help enhance plant growth and modulate the plant response (Backer et al., 2018). Among effective rhizobacteria, two isolates of the infested rhizosphere and all three rhizobacteria of the uninfested rhizosphere were effective phosphate solubilizers. This trait is essential for solubilizing precipitated and fixed phosphorous in various soil types and transforming them into plant-usable forms (Kashyap et al., 2021). In addition, rhizobacteria supply nitrogen to host plants, promoting root and shoot elongation and biomass (Ruzzi&Aroca, 2015). Three rhizobacteria from uninfested rhizosphere,

Table 1: Plant growth-promoting traits associated with the three most effective nematode antagonistic rhizobacteria

Rhizobacteria isolated from infested rhizosphere	P solubilisation	IAA production	Ammonia production
Microbacterium laevaniformans MlIRh3	+	+	-
Staphylococcus kloosii SkIRh4	+	-	+
Priestia aryabhattai PaIRh7	-	+	+
Rhizobacteria isolated from uninfested rhizosphere	**P solubilisation**	**IAA production**	**Ammonia production**
Staphylococcus sciuri SsHRh3	+	-	+
Bacillus pumilus BpHRh5	+	-	+
Priestiamegaterium PmHRh10	+	+	+

(+) = positive, (-) = Negative

Table 2: Substrate utilization profile of nematode antagonistic rhizobacteria

Substrates	*MllRh3*	*SklRh4*	*PalRh7*	*SsHRh3*	*BpHRh5*	*PmHRh10*
Sucrose	-	-	-	-	-	-
Xylose	+	+	+	-	-	-
Maltose	-	+	+	+	-	-
Fructose	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Galactose		-	+	-	-	-
Raffinose	-	+	+	+	+	+
Trehalose		-	+	-	-	-
Melibiose	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	-
Mannose	+	+	-	+	+	+
Inulin	-	-	+	+	-	-
Sodium gluconate	-	-	-	-	-	-
Glycerol	-	-	+	+	-	-
Salicin	+	+	-	+	+	+
Dulcitol	-	-	-	-	-	-
Inositol	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+
Adonitol	-	-	-	-	-	-
Arabitol	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-
alpha-Methyl-D-glucoside	+	-	-	-	-	-
Rhamnose	-	-	-	-	-	-
Cellobiose	+	-	-	+	+	+
Melezitose	+	-	+	+	-	-
alpha-Methyl-D-Mannoside	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-
ONPG	+	+	+	-	+	+
Esculin	+	+	+	+	+	+
D-Arabinose	-	+	-	+	-	-
Citrate	+	+	+	+	+	+
Malonate	-	+	-	+	+	+
Sorbose	-	-	-	-	-	-
Control	-	-	-	-	-	-
Total	15	16	17	18	14	13

(+) = positive, (-) = Negative, MllRh3: *Microbacterium laevaniformans*, SklRh4: *Staphylococcus kloosii*, PalRh7: *Priestia aryabhattai*, SsHRh3: *Staphylococcus sciuri*, BpHRh5: *Bacillus pumilus*, PmHRh10: *Priestia megaterium*.

i.e., *S. sciuri* SsHRh3, *B. pumilus* BpHRh5, *P. megaterium* PmHRh10, and two isolates, i.e., *S. kloosii* SkIRh4, and *P. aryabhattai* PaIRh7 from infested rhizosphere showed the ability to produce ammonia, an essential trait linked to plant growth promotion. Nematode antagonists, *P. megaterium* PmHRh10, *M. laevaniformans* MlIRh3, and *P. aryabhattai* PaIRh7 synthesized IAA in the presence of tryptophan. IAA promotes root development and assists in the uptake of nutrients (Carrillo et al., 2002).

In the current study, thirty-five tests were employed to profile the substrate utilization of effective rhizobacteria. The substrate utilization profile varied among the rhizobacteria, and each isolate utilized a wide range of substrates. Among all, *S. sciuri* SsHRh3 utilized maximum substrates (18) followed by *P. aryabhattai* PaIRh7, *S. kloosii*SkIRh4, *M. laevaniformans*MlIRh3,*B. pumilus* BpHRh5 and *P. megaterium* PmHRh10 which were able to utilize 17, 16, 15, 14 and 13 substrates, respectively (Table 2). Carbon is the primary

Table 3: Effect of nematode antagonistic rhizobacterial isolates and their consortia on tomato plant growth under pot conditions

Treatments	*Shoot length (cm)*	*Fresh shoot weight (g)*	*Root length (cm)*	*Fresh root weight (g)*
T_1	149.4±1.5^{e} (0.0)	288.0±15.6^{e} (0.0)	28.1±0.8^{e} (0.0)	30.1±0.4^{e} (0.0)
T_2	158.8±1.5^{cd} (6.3)	318.2±16.3^{bcde} (10.4)	31.4±0.8^{c} (11.7)	37.4±0.9^{ab} (24.4)
T_3	149.8±1.6^{e} (0.3)	295.1±14.8^{e} (2.4)	28.7±0.8^{e} (2.3)	30.5±0.5^{e} (1.3)
T_4	156.0±1.8^{d} (4.4)	330.0±8.5^{abcde} (14.6)	34.1±0.8^{b} (21.4)	37.4±0.9^{ab} (24.3)
T_5	149.4±1.8^{e} (0.0)	300.2±24.5^{e} (4.2)	29.2±0.5^{de} (4.1)	35.2±0.9^{bcd} (17.1)
T_6	161.0±1.5^{bc} (7.8)	350.0±14.1^{abcd} (21.5)	34.2±0.5^{b} (21.6)	35.5±1.1^{abcd} (17.8)
T_7	157.8±2.2^{cd} (5.6)	310.8±16.7^{cde} (7.6)	31.0±0.4^{cd} (10.3)	35.9±1.0^{ab} (19.3)
T_8	165.0±1.4^{ab} (10.4)	370.0±22.8^{ab} (28.5)	34.8±0.5^{b} (23.8)	36.2±0.7^{ab} (20.2)
T_9	159.8±0.4^{cd} (7.0)	320.2±22.8^{bcde} (11.1)	32.9±0.7^{bc} (17.1)	32.6±0.4^{de} (8.2)
T_{10}	158.8±1.5^{cd} (6.3)	340.0±16.7^{abcde} (18.1)	34.5±0.6^{b} (22.8)	35.7±0.7^{ab} (18.5)
T_{11}	155.6±0.7^{d} (4.1)	340.0±16.7^{abcde} (18.1)	34.6±0.6$^{b\backslash}$ (23.1)	37.5±1.2^{abc} (24.5)
T_{12}	161.4±2.3^{bc} (8.0)	350.5±14.1^{abcd} (21.5)	33.5±0.3^{b} (19.3)	35.4±0.5^{abc} (17.6)
T_{13}	161.8±0.8^{abc} (8.3)	340.5±16.7^{abcde} (18.1)	33.1±0.4^{bc} (17.7)	34.8±0.3^{bcd} (15.6)
T_{14}	161.0±1.5^{bc} (7.8)	360.0±16.7^{abcde} (25.0)	34.2±1.1^{b} (21.6)	32.6±1.6^{cde} (8.4)
T_{15}	166.4±1.2^{a} (11.4)	380.0±22.8^{a} (31.9)	37.9±0.7^{a} (34.9)	38.0±0.9^{a} (26.4)
df	14, 60	14, 60	14, 60	14, 60
F value	9.73	1.91	12.92	6.52
p-value (<0.05)	<0.0001	0.042	<0.0001	<0.0001

Data presented in Mean ± SE. Different letters on each column indicate statistically significant differences between treatments at $P < 0.05$ using Tukey's HSD test. T_1- Untreated control (40 ml /plant); T_2- *Microbacterium laevaniformans* (MlIRh3) @ 40 ml/plant; T_3- *Staphylococcus kloosii* (SkIRh4) @ 40 ml/ plant; T_4- *Priestia aryabhattai* (PaIRh7) @ 40 ml/ plant; T_5- MlIRh3 + SkIRh4 @ 40 ml/ plant; T_6- MlIRh3 + PaIRh7 @ 40 ml/ plant; T_7- SkIRh4 + PaIRh7 @ 40 ml/plant; T_8- MlIRh3 + SkIRh4 + PaIRh7@ 40 ml/ plant; T_9- *Staphylococcus sciuri* (SsHRh3) @ 40 ml/plant; T_{10}- *Bacillus pumilus*(BpHRh5) @ 40 ml/plant; T_{11}- *Priestia megaterium* (PmHRh10) @ 40 ml/plant; T_{12}- SsHRh3 + BpHRh5 @ 40 ml/ plant; T_{13}- SsHRh3 + PmHRh10 @ 40 ml/ plant; T_{14}- BpHRh5 + PmHRh10 @ 40 ml/ plant; T_{15}- SsHRh3 + BpHRh5 + PmHRh10 @ 40 ml/ plant.

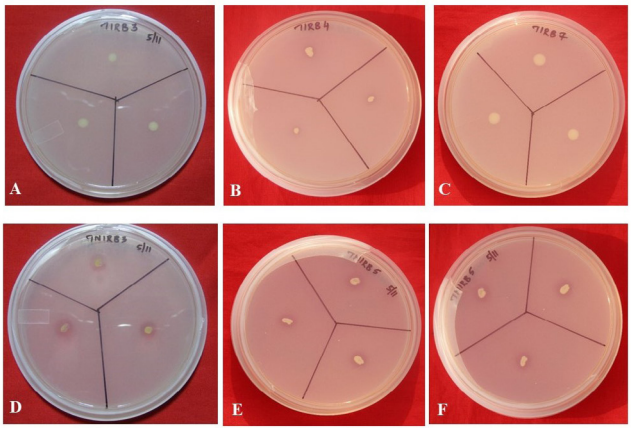


Figure 1: Phosphate solubilisation activity of rhizobacterial isolates A. *Microbacterium laevaniformans* IRh3 B. *Staphylococcus kloosii* IRh4 C. *Bacillus aryabhattai* IRh7, D. *Staphylococcus sciuri* HRh3, E. *Bacillus pumilus* HRh5, F. *Priestia megaterium* HRh10

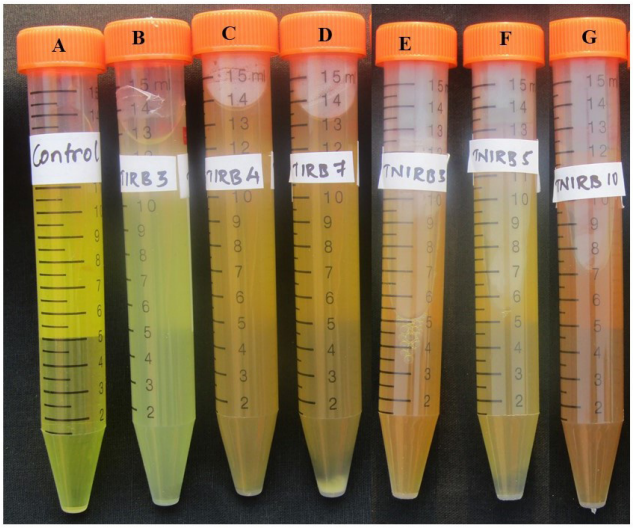


Figure 2: Ammonia production from rhizobacterial isolates A. Control B. *Microbacterium laevaniformans* IRh3 C. *Staphylococcus kloosii* IRh4 D. *Bacillus aryabhattai* IRh7, E. *Staphylococcus sciuri* HRh3, F. *Bacillus pumilus* HRh5, G. *Priestia megaterium* HRh10. Substrate utilization profile of nematode antagonistic rhizobacterial isolates

energy source for microorganisms. It plays a crucial role in microbial growth and the production of primary and secondary metabolites (Singh et al., 2017). The choice of carbon source greatly influences secondary metabolism (Marwick et al., 1999). Thus, knowing the metabolic requirements of a bacterium can lead to a better understanding of the conditions under which it is likely to proliferate and generate suitable biomass (Scaria et al., 2014). *S. sciuri* SsHRh3 from the uninfested rhizosphere could utilize maximum substrates, followed by *P. aryabhattai* from the infested rhizosphere. Antibiotic production by rhizobacterial strains is closely related to cell metabolic status. It is highly influenced by biotic and abiotic stimuli (Raaijmakers et al., 2002), including the type of substrates present in a particular habitat, host plant growth, temperature, oxygen availability, and pH. Multiple substrate utilization ensures better survival and establishment of rhizobacteria, thereby antagonistic activity against nematodes. Moreover, modulation of the populations of different species of bacteria in an environment would depend on substrate availability, its utilization by the microorganisms, and conversion to the other forms in a manner that allows their coexistence.

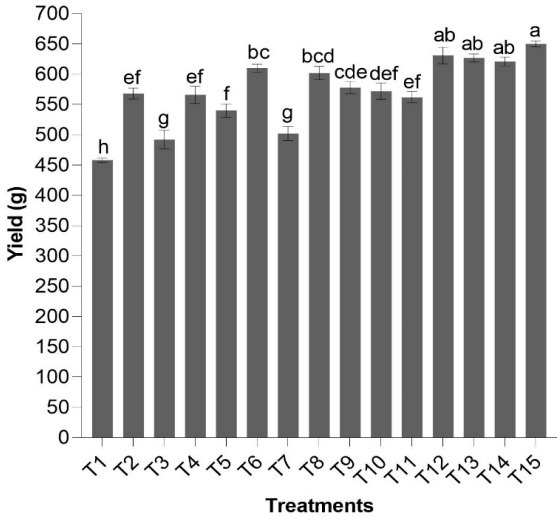
Effect of Nematode Antagonistic Rhizobacterial Isolates on Tomato Plant Growth under Pot Conditions

In a previous study, drenching of rhizobacteria either singly or consortia significantly reduced nematode reproduction in tomato plants. Application of rhizobacteria reduced the number of galls per root system in the range of 45.4 to 80.6%, egg mass per root system in the range of 34.4 to 80.5% and soil nematode population at the harvest in the range of 25.1 to 58.2% compared to untreated control. Among rhizobacteria treatments, the consortium of *S. sciuri* SsHRh3 + *B. pumilus* BpHRh5 + *P. megaterium* PmHRh10 isolated from healthy tomato rhizosphere was most effective in reducing nematode reproduction in tomato plants. However, *S. kloosii* SkIRh4 and its consortium with other rhizobacteria from root-knot nematode-infested tomato rhizosphere were the least effective (Gowda et al., 2023).

In the present study, drenching of rhizobacteria either singly or in consortia on tomato plants resulted in a significant effect on tomato plant growth. Either singly or consortia application of rhizobacterial isolates have increased root length in the range of 2.3 to 34.9%, fresh root weight 1.1 to 36.9%, shoot length 0.0 to 11.4%, fresh root weight 1.3 to 26.4%, and yield from 7.4 to 41.9%. From 15 treatments, shoot and root length of 12 treatments, fresh root weight of 11 treatments, fresh shoot weight of 04 treatments, and yield of 14 treatments were significantly higher than untreated control (Table 3, Figure 4). Among rhizobacterial treatments, the consortium of three rhizobacterial isolates (T_{15}) from an uninfested rhizosphere and T_8 (a consortium of three rhizobacterial isolates from an infested rhizosphere) greatly enhanced plant growth parameters and yield. In addition, the consortia, SsHRh3 + BpHRh5, SsHRh3 + PmHRh10, and BpHRh5 + PmHRh10 and MlIRh3 + PaIRh7 were the next most effective rhizobacterial treatments with respect to enhancement of tomato plant growth.

Analysis of variance revealed that root length (df: 14, 60, F value: 12.92, p<0.0001), fresh root weight (df:14, 60, F value: 6.52, p <0.0001), shoot length (df: 14, 60, F value: 9.73, p<0.0001), fresh shoot weight (df: 14, 60, F value: 1.91, p: 0.042) and yield (df: 14, 60, F value: 22.68, p<0.0001) was significantly enhanced in the rhizobacteria treatments compared to untreated control (Table 3 and Fig. 1). Besides, tomato root length and fruit yield was considerably increased in plants treated with the consortia of rhizobacteria from uninfested rhizosphere than

Figure 3: Substrate utilization profile of rhizobacterial isolates A. *Microbacterium laevaniformans* IRh3 B. *Staphylococcus kloosii* IRh4 C. *Bacillus aryabhattai* IRh7, D. *Staphylococcus sciuri* HRh3, E. *Bacillus pumilus* HRh5, F. *Priestia megaterium* HRh10



Different letters on error bars indicate statistically significant differences between treatments at $P < 0.05$ using Tukey's HSD test. T_1- Untreated control (40 ml /plant); T_2- *Microbacterium laevaniformans* (MIIRh3) @ 40 ml/plant; T_3- *Staphylococcus kloosii* (SkIRh4) @ 40 ml/ plant; T_4- *Priestia aryabhattai* (PaIRh7) @ 40 ml/ plant; T_5- MIIRh3 + SkIRh4 @ 40 ml/ plant; T_6- MIIRh3 + PaIRh7 @ 40 ml/ plant; T_7- SkIRh4 + PaIRh7 @ 40 ml/plant; T_8- MIIRh3 + SkIRh4 + PaIRh7@ 40 ml/ plant; T_9- *Staphylococcus sciuri* (SsHRh3) @ 40 ml/ plant; T_{10}- *Bacillus pumilus*(BpHRh5) @ 40 ml/plant; T_{11}- *Priestia megaterium* (PmHRh10) @ 40 ml/plant; T_{12}- SsHRh3 + BpHRh5 @ 40 ml/ plant; T_{13}- SsHRh3 + PmHRh10 @ 40 ml/ plant; T_{14}- BpHRh5 + PmHRh10 @ 40 ml/ plant; T_{15}- SsHRh3 + BpHRh5 + PmHRh10 @ 40 ml/ plant.

Figure 4: Effect of root-knot nematode antagonistic rhizobacterial isolates and their consortia on tomato yield under pot conditions

rhizobacteria consortia of nematode infested rhizosphere (Table 3 and Fig. 1). Besides, application of rhizobacteria, i.e., SkIRh4 either singly or in consortia, no significant effect on plant growth was observed under the pot experiment. Similar to our study, a consortium of Indigenous bacteria, i.e., *Bacillus* sp. + *S. sciuri* and *Bacillus* sp. + *Ochrobactrum* sp. + *B. cereus* + *S. sciuri* isolated from healthy tomato root surface was significantly effective in enhancing plant growth as well as nematode suppression in tomato plants under pot conditions (Alfianny et al., 2017). In comparison to rhizobacteria from both the environments, better yields of tomatoes and root length were observed when the plants were treated with a consortium of rhizobacteria from uninfested rhizosphere than rhizobacterial-consortia of nematode-infested rhizosphere. Thus, the selection of promising rhizobacteria or a consortium based on potential nematicidal activity along with associated plant growth promotion traits helps in greater suppression of PPNs in various crops.

Conclusion

In summary, the present study revealed that nematode antagonistic rhizobacteria except *S. kloosii*SkIRh4 proved as potential candidates for PGP activity by contributing a significant effect on tomato plant growth. Thus, the utilization of promising rhizobacteria either singly or in consortia can serve as safe alternative synthetic chemical nematicides for the suppression of root-knot

nematode incidence in tomatoes grown under a protected environment. Nevertheless, further evaluation is needed to test the nematicidal potential with PGP activity under field conditions prior to considering them as a component in root-knot nematode-integrated management strategies.

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सारांश

वर्तमान अध्ययन में, जन्मज जड़-गांठ सूत्रकृमि प्रतिपक्षी राइजोबैक्टीरिया यथा, माइक्रोबैक्टीरियम लेवेनिफोर्मन्स डसप्ती3, स्टैफिलोकोकस क्लोसीाप्ती4, और प्रीस्टिया आर्यभट्टई चंप्ती7 को मेलोइडोगाइन इन्कॉग्निटा संक्रमित और स्टैफिलोकोकस साइउरीैभ्ती3 बैसिलस प्यूमिलस ठचभ्ती5, प्रीस्टिया मेगाटेरियम च्उभ्ती10 को संरक्षित संरचनाओं में उगाये गए असंक्रमित टमाटर के पौधे से पृथक कर पादप विकास को बढ़ावा देने वाले गुणों (जैसे, फॉस्फेट घुलनशीलता गतिविधि, इंडोल एसिटिक अम्ल उत्पादन, अमोनिया उत्पादन और क्रियाधार (सब्सट्रेट) उपयोग प्रालेख) और मिट्टी में उत्तरजीविता के गुणों का लक्षण वर्णन किया गया है। राइजोबैक्टीरिया में, स्टैफिलोकोकस साइउरीैभ्ती3 बैसिलस प्यूमिलस, ठचभ्ती5 प्रीस्टिया मेगाटेरियम च्उभ्ती10 माइक्रोबैक्टीरियम लेवेनिफोर्मन्स डसप्ती3 और स्टैफिलोकोकस क्लोसीाप्ती4 ने पिकोवास्काया वृद्धि माध्यम पर फॉस्फेट घुलनशीलता गतिविधियाँ दिखाईं। ट्रिप्टोफैन की उपस्थिति में, राइजोबैक्टीरिया प्रीस्टिया मेगाटेरियम च्उभ्ती10, माइक्रोबैक्टीरियम लेवेनिफोर्मन्स डसप्ती3, और प्रीस्टिया आर्यभट्टई चंप्ती7 ने इंडोल एसिटिक एसिड का उत्पादन किया। इसी तरह, स्टैफिलोकोकस साइउरीैभ्ती3ए बैसिलस प्यूमिलस, ठचभ्ती5ए प्रीस्टिया मेगाटेरियम च्उभ्ती10ए स्टैफिलोकोकस क्लोसीाप्ती4, और प्रीस्टिया आर्यभट्टई चंप्ती7 ने प्रयोगशाला स्थितियों के अंतर्गत अमोनिया का उत्पादन किया। अध्ययन किए गए 35 क्रियाधार में से, राइजोबैक्टीरिया में अलग-अलग क्रियाधार उपयोग प्रालेख थे, स्टैफिलोकोकस साइउरीैभ्ती3 ने अधिकांश क्रियाधार (18) का उपयोग किया, तत्पश्चात पी प्रीस्टिया आर्यभट्टई चंप्ती7ए स्टैफिलोकोकस क्लोसीाप्ती4, माइक्रोबैक्टीरियम लेवेनिफोर्मन्स डसप्ती3, बैसिलस प्यूमिलस ठचभ्ती5 और प्रीस्टिया मेगाटेरियम च्उभ्ती10 जो क्रमशः 17, 16, 15, 14 और 13 क्रियाधर का उपयोग करने में सक्षम थे। इसके अतिरिक्त, इन राइजोबैक्टीरियल व्यावर्तकों के एकल या कंसोर्टिया में उपयोग से ज्ञात होता है कि, सूत्रकृमि असंक्रमित मूल परिवेश से सभी तीन राइजोबैक्टीरियल व्यावर्तकों यानीैभ्ती3. ठचभ्ती5. च्उभ्ती10 के एक कंसोर्टियम ने अनुपचारित नियंत्रण और एकल अनुप्रयोग की तुलना में उच्चतम पौधों की वृद्धि को बढ़ावा देने वाली गतिविधि दर्ज की है। इसके विपरीतए स्टैफिलोकोकस क्लोसीाप्ती4 का अकेले या कंसोर्टिया में, गमले में किए गए अध्ययन के अंतर्गत पौधों की वृद्धि पर कोई महत्वपूर्ण प्रभाव नहीं देखा गया। कुल मिलाकर, अध्ययन में पाया गया कि जड़-गांठ सूत्रकृमि प्रतिपक्षी राइजोबैक्टीरियल व्यावर्तक पादप वृद्धि को बढ़ाने में आशाजनक परिणाम दिये। संरक्षित वातावरण में उगाए गए टमाटरों में जड़-गांठ सूत्रकृमि के संक्रमण को दबाने के लिए इन्हें अकेले या संयोजन में उपयोग करना कृत्रिम रासायनिक सूत्रकृमिनाशियों का एक सुरक्षित विकल्प हो सकता है।