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Nematicidal rhizobacteria with plant growth-promoting traits associated with tomato in root-knot infested polyhouses

M. Devindrappa¹, Anju Kamra^{1*}, Minakshi Grover² and Bharat Gawade³

Abstract

Background: In polyhouse cultivation of tomato (*Solanum lycopersicum*) in root-knot (*Meloidogyne incognita*) infested soils, several accessions remained free of nematode infection. It was hypothesized that such plants that were free of root-knot infection in the selected polyhouses were possibly harboring potent nematode antagonistic microbes. Therefore, attempts were made to isolate root-associated rhizobacteria from nematode infected and uninfected roots of 7 tomato accessions. Eighty morphologically distinct isolates were multiplied in nutrient broth for 48 h to test the presence of nematotoxic metabolites. Laboratory bioassays were conducted using their cell-free filtrates to observe mortality in *M. incognita* juveniles (J2s) at 24 h. The most potent ones were identified through molecular characterization (16S rDNA) and assayed for plant growth promotion traits, seed germination and seedling vigor and bioefficacy against *M. incognita* infecting polyhouse tomato.

Results: Rhizobacterial densities were estimated from the root samples using 3 media. In all the media, the average numbers of colony forming units (cfu) from uninfected roots were significantly higher than the infected roots. The maximum numbers of colonies were observed in soil plate count agar. The extracellular metabolites caused juvenile mortality in the range of 60.67–100% from isolates associated with nematode uninfected roots of accessions H308, H299 and H266, as compared to isolates from nematode infected roots of accessions H195, BSS99, H178/F4 and H88, which caused mortality in the range of 52.33 to 73.67%. Four isolates (B9, B16, B26 and B31) caused 100% J2 mortality, followed by 13 isolates (B7, B8, B13, B14, B17, B18, B21, B22, B25, B28, B29, B32 and B33) that caused 90–95%, 12 isolates caused 80–85% and 51 isolates caused < 85% J2 mortality, when exposed to bacterial metabolites in cell-free filtrates for 24 h. The isolates B9, B16, B26 and B31 were identified based on 16S rRNA sequence analysis as *Bacillus pumilus, B. megaterium, B. subtilis* and *B. cereus*, respectively. These 4 bacterial isolates possessed plant growth promotion traits like production of IAA, ammonia, catalase and chitinase with the ability to solubilize zinc and phosphate. These enhanced tomato seed germination and seedling vigor, and their application in soil resulted in significant increase in root and shoot length of tomato seedlings.

Conclusions: In the present investigation, the 21% bacterial colonies (17 isolates) that caused nemato toxicity in the range of 90 to 100% and another 15% (12 isolates) that caused juvenile mortality in the range of 80 to 90% were from uninfected tomato accessions, indicating their role in protecting the plants against root-knot nematode (RKN) infection. The 4 bacterial isolates characterized from uninfected plants possessed high nematicidal potential with plant

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growth promotion (PGP) traits that enhanced tomato seed germination and seedling vigor index. The isolates can be utilized for root-knot nematode pest management in polyhouse cultivation.

Keywords: Rhizobacteria, *Meloidogyne incognita, Bacillus* spp., Extracellular metabolites, Plant growth-promoting traits

Background

In India, the National Horticulture Mission enacted the promotion of polyhouse and greenhouse; presently it occupies an area of 30,000 ha cultivating vegetables and ornamental crops (Reddy 2016). Though the cultivation of crops in polyhouses enhances the yield per unit area, the altered environment promotes the build-up of soil-borne pests and pathogens. RKN species, Meloidogyne incognita and M. javanica, are serious constraints to vegetable crops, especially tomato (Solanum lycopericum) under polyhouse cultivation (Phani et al. 2021) causing yield losses by 25-100% (Seid et al 2015). Application of chemical nematicides is the most common short-term management strategy for the nematode pest. However, several chemical nematicides are withdrawn from the market due to environmental concerns (Xiang et al. 2018). With increasing awareness on environmental protection and food safety, there is a need to exploit the microbial resources present in the rhizosphere. Variable infestation of the nematode is often observed in plants of the same genotype due to differences in the soil microbiome. The rhizosphere bacteria like Bacillus subtilis, B. licheniformis, B. megaterium, B. coagulans and Pseudomonas fluorescens directly and indirectly suppressed *M. incognita* due to nematicidal metabolites released by them (Xiang et al 2018). Various Bacillus spp. stimulated the plant growth promotion, enhanced acquisition of nutrients and excited plant defense mechanism against soil-borne pathogens and nematodes (Alooa et al. 2019). The composition and function of microbial communities in the rhizosphere can play a vital role in plant growth and nematode infection. Though the soil microbiome effects on root-knot infection using 16s RNA genes of bacterial communities have been reported for open field conditions (Zhou et al. 2019), the identification of culturable nematode antagonistic bacteria associated with uninfected roots in polyhouses has not been reported. In the present study, it was hypothesized that tomato plants free of root-knot infection in the selected polyhouses are harboring more nematode antagonistic bacteria. Therefore, root-associated rhizobacteria from nematode infected and uninfected tomato in polyhouse cultivation were isolated and evaluated for nematode antagonism. The most potent ones were identified through molecular characterization and assayed for plant growth promotion characters.

Methods

Isolation of root associated rhizobacteria from tomato crop Sample collection and isolation of rhizobacteria

Three polyhouses at ICAR-Centre for Protection Cultivation Technology, Indian Agriculture Research Institute (IARI), New Delhi, with root-knot infestation, were selected for sampling. Root samples were collected in December, from infected and uninfected 7 accessions of tomato. Five root samples from each row were pooled to form one composite sample, which was labeled and stored in plastic bags at 4 °C until processed. Whole plants were uprooted from the soil to examine nematode infection. Infected roots had heavy galling, as compared to uninfected roots that had very few galls or no galls. For the isolation of bacteria, 10 g of tomato roots was cut into small pieces and added to 90 ml of 0.85% sodium chloride (dilution 10^{-1}) and shaken at 200 rpm for 1 h in an orbital shaker (New Brunswick Scientific, USA). Suspensions were serially diluted with sterile distilled water to prepare 10^{-4} dilutions, agitated and a 100 µl of suspension was plated on 3 nutrient media, viz. Soil Plate Count Agar (SPCA), Soil Extract Agar (SEA) and King's B procured from Hi Media, Mumbai, India. Plates were incubated for 12-48 h. in a BOD incubator at 30 °C using 5 replicates. The numbers of bacterial colonies were counted under a stereoscopic microscope at 10x. The morphologically distinct bacterial colonies were isolated based on shape, size, elevation, opacity and margin and multiplication on nutrient broth (NB) for nematicidal evaluation. The selected bacterial isolates were sub-cultured to purify and maintained in 15% glycerol vials, at -80 °C.

In vitro screening of rhizobacteria from infected and uninfected plants for juvenile mortality

Freshly prepared NB was inoculated with the selected colonies and incubated on a shaker at 120 rpm for 48 h, followed by centrifugation at 10,000 rpm for 10 min. The supernatants were filtered using Whatman No.1 paper, followed by nitrocellulose syringe filter (0.20 μ m) to obtain cell-free filtrates (CFF) with metabolites if any. The egg masses of *M. incognita* were hand-picked from culture pots maintained at greenhouse, Division of Nematology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. The *Meloidogyne* species was identified based on the perineal pattern structure of female nematode (Taylor et al. 1955).The egg masses were

kept for hatching at 21° C in a BOD incubator. A 1 mL of CFF was pipetted in each well of pre-sterilized multiwell plates, followed by addition of 100 J2s of freshly hatched *M. incognita* in 100 μ l water and incubated at 25 °C, with sterile medium and sterile distilled water (SDW) as control treatments. The immobile J2s were counted at 24 h under a stereoscopic zoom binocular microscope and then transferred to distilled water for 24 h to confirm their mortality. Five replicates were maintained for each isolate.

Molecular Characterization of rhizobacterial isolates

The 24-h bacterial cultures were used for genomic DNA isolation by Zymo Research Crop quick DNA [™] Fungal/ Bacterial Miniprep kit (Cat. No. D6005) according to manufacturer's instructions and analyzed by 0.8% agarose gel. The purity and quantity of the DNA were checked in Nanodrop spectrophotometer (Thermo scientific, USA). The DNA yield was quantified in ng mL $^{-1}$, and the purity was assessed at 260/280 nm absorbance ratio. The PCR amplifications of 16S rRNA was performed using the universal primers were 27F (5-GTTTGATCCTGGCTCAG-3) and 1494R (5-ACGGCTACCTTGTTACGACTT-3) used for 16S rRNA gene amplification. The PCR was carried out using standardized protocol, and the amplicons were sent for Sanger sequencing to ABA Biotech Pvt. Ltd. The nucleotide sequences obtained were aligned with the existing nucleotide database of NCBI GenBank, and the reference sequences were retrieved. Phylogenetic tree was constructed using MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) software to understand the phylogenetic relationship of the selected 4 isolates. The nucleotide sequences were compared against nucleotide database using the NCBI BLASTn and EzTaxon SERVER 2.1 programs to identify the closest known taxa. The 16S rRNA gene along with the closest homology sequences was aligned using multiple sequence alignment program CLUSTAL W algorithm implemented in MEGA 6 SOFTWARE using default parameters. The phylogenetic tree was constructed by neighbor joining method, using MEGA 6 PROGRAM, and evolutionary distances were compared with the help of Kimura's parameter models. The 16S rRNA gene sequences obtained were submitted under accession numbers MZ675428-MZ675431.

Biochemical assays for plant growth-promoting (PGP) traits

Indole acetic acid (IAA) production (Gordon and Weber 1951) The IAA was estimated using 48-h bacterial cultures grown in NB at 30 °C. The cultures were centrifuged at 10,000 rpm for 10 min, and supernatant (4 ml) was mixed with 2 drops of ortho-phosphoric acid and 4 ml of Salkowski reagent (48 mL of 35% perchloric acid and

2 mL of 0.5 M ferric chloride solution) in 1:2 ratio (bacterial supernatant/reagent) at room temperature. The appearance of pink color indicated production of IAA. The optical density was observed at 530 nm after 25 min in 96-well microtiter plates using multimode reader (Thermo scientific, USA).

Phosphate and zinc solubilization test (Pikovaskya 1948)

The estimation of phosphate solubilization was conducted by spotting the bacterial cultures on the Pikovaskya agar plates and incubated at 30 °C for 5 days. The appearance of clear zone around the colony was considered positive for phosphate solubilization. Similarly, 24-h-old bacterial culture was inoculated on Tris mineral salts agar medium amended with 0.2% soluble zinc oxide (ZnO) and incubated for 3–5 days at 30 °C. The Zn solubilization was determined by appearance of clear zone around the bacterial colony (Gaur 1990).

Hydrogen cyanide (HCN) production (Bakker and Skipper 1987)

The 24-h-old bacterial cultures were inoculated on nutrient agar amended with glycine (0.44%) by spread plate method. Whatman No. 1 filter paper pre-saturated with 0.5% picric acid in 2% sodium carbonate solution (w/v) was placed in the lid of Petri plate, and the plates were sealed with parafilm (to prevent leakage), followed by incubation at 30 °C for 3–5 days. The HCN production was observed by change in color of filter paper from yellowish brown to deep brown.

Catalase production test (Schaad 1992)

The catalase test was performed by adding drop of 3% hydrogen peroxide to 48-h bacterial colony on a clean glass slide and mixed through sterile toothpick. The appearance of effervescence indicated catalase production.

Chitinase production test (Rodriguez-Kabana et al. 1983)

The M9 Agar media amended with 1% (w/v) colloidal chitin was prepared and plates were divided into 4 equal sectors, followed by spot inoculation with 24-h-old bacterial cultures and incubation at 30 °C for 5 days. The appearance of clearance zone around the bacterial colonies indicates chitinase production.

Ammonia (NH3) production test (Cappuccino and Sherman 1992)

The overnight bacterial culture (100 μ l) was mixed, inoculated into 10 ml peptone water and incubated at 30 °C for 4 days. After incubation, an 1 mL of culture broth was added with 1 ml Nessler's reagent. Formation of a yellow

to deep orange color was considered positive for ammonia production.

Seed germination test

In the seed germination test, by paper towel method, the bacterial isolates B9, B16, B26 and B31 were used for seed treatment of tomato variety Pusa Ruby. Seeds were surface sterilized with 0.1% HgCl2 (5 min), washed 4–5 time with sterile distilled water and soaked in liquid broth culture (10^8 cells/mL) for 1 h. Each seed was placed on wet blotting paper using sterile forceps, rolled the paper and incubated in BOD for 16 days at 30 °C. Seeds treated with sterile nutrient broth and distilled water served as control, and 5 replications for each isolate were maintained. Germination percentage and seedling vigor index were assessed as per protocols of International Seed Testing Association (1985).

Bioefficacy of rhizobacteria against M.*incognita* infecting tomato cv Pusa Ruby

The bioefficacy trial was conducted in 10 inch pre-sterilized plastic pots using 2-week-old seedlings of tomato cv Pusa Ruby transplanted in double sterilized field soil mixed with 3% bacterial cultures (*B. pumilus. B. megaterium, B. subtilis* and *B. cereus*) @ ~10⁸ cfu/mL. Freshly hatched *M. incognita* was inoculated @ 2J2/cc soil after 7 days of transplanting. The nematicide velum prime (VP) from Bayer Pvt. Ltd. was taken as an additional treatment for comparison and applied (0.56µL/kg soil) after mixing in 500 mL sterilized water for uniform spread. The treatments were T1: Sterilized Water, T2: Sterilized Water + Mi, T3: *B. pumilus*, T4: *B. pumilus* + Mi, T5: *B. megaterium*, T6: *B. megaterium* + Mi, T7: *B. subtilis*, T8: *B. subtilis* + Mi, T9: *B. cereus*, T10: *B. cereus* + Mi, T11: Media (NB), T12: VP (velum prime), and T13: VP + Mi. Six replications of the 13 treatments were maintained in a polyhouse for 75 days. The plants were uprooted for recording observations on number of root galls per root, number of J2 s per cc soil, number of egg masses per root, number eggs per egg mass, nematode reproduction factor and plant growth parameters.

Statistical analysis

Data of colony forming units of bacterial isolates from infected and uninfected plants were analyzed for significant mean differences via one-way ANOVA using statistical software Statistical Tool for Agricultural Research (STAR). The multiple mean comparisons were performed using Tukey's honestly significant difference test (Tukey's HSD).

Results

Isolation, selection and evaluation of bacterial isolates for nematicidal activities

The bacterial colonies from root-knot infected and uninfected tomato roots in 7 accessions produced distinct colonies in the range of 40.4 to 91.6×10^4 /g root in the 3 media (Table 1). Among the 3 media, SPCA gave significantly (P < 0.05) higher number of colonies, followed by Kings B and SEA for both nematode uninfected and infected roots. The bacterial colonies were significantly higher in nematode uninfected tomato roots than the nematode infected roots in all the 7 accessions of tomato. On an average, 78–91 cfu × 10⁴ bacterial/g root was found in nematode uninfected roots, compared to 40-54 cfu × 10^4 /g root in nematode

 Table 1
 Root-associated rhizobacterial densities in root-knot nematode (RKN) infected and uninfected tomato in polyhouse cultivation

Culture media	Isolation source				
	RKN uninfected to	omato roots	RKN infected tom	ato roots	Total isolates selected
	$CFU g^{-1}$ root $ imes 10^{4*}$	Distinct selected colonies	$CFU g^{-1}$ root $\times 10^{4*}$	Distinct selected colonies	
SPCA	91.6 ± 2.6^{a}	20.00	54.6 ± 2.5^{a}	13.00	33.00
SEA	78 ± 2.4^{b}	10.00	40.4 ± 3.3^{b}	12.00	22.00
Kings B	84 ± 1.3^{b}	15.00	46.4 ± 2.4^{ab}	10.00	25.00
CD	2.2		2.2		
<i>F</i> Value	2322.67		846.89		
Pr (> <i>F</i>)	0.0001		0.0001		

Numerical values are Mean \pm SE, means with the same letter are not significantly different

RKN, root-knot nematode; SPCA, soil plate count agar; SEA, soil extract agar

*Average of 5 replicates

infected roots. Eighty morphologically distinct bacterial isolates were selected from the 3 nutrient media. On screening for juvenile mortality, 4 isolates B9, B16, B26 and B31 caused (100%) J2 mortality, followed by 13 other isolates (B7, B8, B13, B14, B17, B18, B21, B22, B25, B28, B29, B32 and B33) which caused mortality in the range of 90–95% and 12 isolates (B1,B2, B3, B6,B11, B15,B19, B20, B27, B30, B34 and B35) which caused 80–89% mortality. A total of 51 isolates caused <80%



J2 mortality. The mortality bioassays revealed that the most isolates associated with uninfected roots, caused mortality in the range of 80-90%, as compared to isolates from infected roots, where the most cultivable isolates caused juvenile mortality in the range of 61–70% (Fig. 1).

Molecular characterization

The 16S rRNA nucleotide sequence of the 4 isolates, viz. B9, B16, B26 and B31, was analyzed using the BLAST [®] sequence provided by the National Centre for Biotechnology Information (NCBI). These were identified as *Bacillus pumilus, B. megaterium, B. subtilis* and *B. cereus* with GenBank accessions nos. B9-MZ675428, B16-MZ675429, B26-MZ675430 and B31-MZ675431, respectively (Table 2).

Phylogenetic tree

The nucleotide sequences obtained were aligned with the existing nucleotide database of NCBI GenBank and reference sequences were retrieved. Phylogenetic tree was constructed by using MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) software. The phylogenetic tree was found to possess substantial genetic homogeneity among the 4 *Bacillus* isolates (Fig. 2). The isolates may be divided into 4 clades of the phylogenetic tree. Isolates *B. pumilus* and *B. subtilis* came under the same clade, whereas *B. megaterium* and *B.* cereus were in other clade of the phylogenetic tree.

Plant Growth-Promoting (PGP) traits

The isolates, viz. B9, B16, B26 and B31, were found Gram positive and exhibited multiple plant growth promotion traits (Table 3). All isolates showed phosphate production, zinc solubilization ability, HCN, chitin, catalase, ammonia and IAA production.

 Table 2
 Rhizobacterial isolates and their identity based on 16S rRNA gene sequence similarity

S. No	lsolates	Isolation media	NCBI strain	Similarity (%)	GenBank Accession No
1	B9	SEA	Bacillus pumilus	100	MZ675428
2	B16	SPCA	Bacillus megaterium	100	MZ675429
3	B26	SPCA	Bacillus subtilis	100	MZ675430
4	B31	SEA	Bacillus cereus	100	MZ675431

Homology and phylogenetic identity of the rhizobacterial isolates were obtained by comparing the 16S rRNA gene sequence similarity with that of related isolates available at the NCBI database

SPCA, soil plate count agar, this media used for isolation of rhizosphere bacteria; SEA, soil extraction agar

Enhancement of tomato seed germination and seedling vigor index (SVI)

All the selected bacterial isolates enhanced the percentage seed germination and seedling vigor of tomato variety Pusa Ruby than in the untreated control on all 4 days of observation (Table 4). However, the maximum seed germination percentage (97%) and seedling vigor index (1789) were observed in B26 (*B. subtilis*) treatment, followed by B9, B16 and B31.



Table 3 Plant growth-promoting characters of rhizobact	terial isolates
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S. No	Isolates	PS	ZS	СНР	HP	IP	СР	AP
1	Bacillus pumilus (B9)	+	+	+	+	+	+	+
2	Bacillus megaterium (B16)	+	+	+	+	+	+	+
3	Bacillus subtilis (B26)	+	+	+	+	+	_	+
4	Bacillus cereus (B31)	+	+	+	+	+	+	+

PS, phosphate solubilization; ZS, zinc solubilization; CHP, chitinase production; HP, HCN production; IP, IAA production; CP, catalase production; AP, ammonia production (NH₃)

Table 4 Effect of rhizobacterial isolates on tomato seed germination and seedling vigor index at different time interval

Isolates	Seed germi	nation (%)			Seedling Vigor index					
	Days				Days					
	4	8	12	16	4	8	12	16		
Bacillus pumilus (B9)	26.0 ± 1.0^{b}	75.0 ± 1.7^{b}	89.3 ± 1.2^{a}	93.3 ± 0.3^{b}	121.3±0.6	675.0 ± 0.5	1101.7±0.3	1337.7 ± 0.3		
<i>B.megaterium</i> (B16)	$25.6\pm1.2^{\rm b}$	$75.0\pm1.7^{\rm b}$	88.0 ± 2.5^{ab}	$91.3\pm0.6^{\rm b}$	102.6 ± 0.5	750.0 ± 0.5	1261.3 ± 0.3	1370.0 ± 0.5		
B. subtilis (B26)	31.0 ± 0.5^a	86.6 ± 0.8^a	95.6 ± 0.3^{a}	97.0 ± 0.5^a	186.0 ± 0.5	1008.8 ± 0.6	1525.3 ± 0.3	1789.6 ± 0.3		
B. cereus (B31)	$25.3\pm1.4^{\rm b}$	$75.6\pm2.0^{\rm b}$	88.0 ± 3.0^{ab}	$91.0\pm0.5^{\rm b}$	84.4 ± 0.3	780.0 ± 0.5	1275.5 ± 0.3	1455.0 ± 0.5		
Control (media)	$10.0 \pm 1.1^{\circ}$	$68.0\pm2.5^{\rm b}$	$79.0\pm1.0^{\circ}$	$84.6\pm0.3^{\circ}$	16.6 ± 0.3	297.5 ± 0.3	584.2 ± 0.3	963.0 ± 0.3		
Water	$10.0\pm1.1^{\circ}$	$68.6\pm2.4^{\text{b}}$	79.6 ± 0.8^{bc}	$85.0\pm0.5^{\circ}$	16.6 ± 0.3	297.5 ± 0.3	584.2 ± 0.3	991.6 ± 0.3		
<i>F</i> value	74.48	11.77	12.82	83.11						

Numerical values are Mean \pm SE; means with the same letter are not significantly different

DW, distilled water; media, nutrient agar media; seedling vigor index, germination (%) \times seedling length (cm)

Effect of rhizobacteria on plant growth parameters of tomato cv Pusa Ruby

On soil application the 4 rhizobacteria resulted an enhancement in shoot length and weight and fresh biomass. The percent increase in shoot length varied from 13.96 to 28.64, root length from 11.20 to 25.68; fresh shoot weight from 17.88 to 38.05; and fresh root weight from 19.54 to 56.90, in bacteria alone treatments as compared to uninoculated control (Table 5). The maximum enhancement in all the above parameters was observed in B. subtilis treatments. On an average, the plant growth parameters showed the enhanced effect in presence of the nematode, except in root length where a decrease was observed due to twisting of roots due to nematode galling. However, a significant Pusa (P < 0.05) enhancement in root volume was observed with all the rhizobacteria, though the effect was most enhanced with Bacillus subtilis, and that of other rhizobacterial species was at par (Fig. 3). An increase of 88.31 in root volume was observed even in presence of the nematode. Despite some negative effect on root length and weight in the presence of the nematode, the total biomass increased significantly (P < 0.05) an application of rhizobacteria; the effect was the maximum in B. subtilis application, where 43.8% increase was observed even in presence of the nematode. The chemical nematicide velum prime did not show an increase in shoot length and weight and caused reduction in root length and weight in presence of nematode, though a 2.58% increase in biomass was observed.

Effect on rhizobacteria on nematode infection

An average of 263 galls per plant was developed 75 days after nematode inoculation which reduced significantly (P<0.05) in presence of B. pumilus (41.5), B. megaterium (42.83), B. subtilis (21.17) and B. cereus (44.3). Thus, the reduction was significant an application of rhizobacteria. The average number egg mass per plant was 162.83 in untreated control which reduced in presence of B. pumilus (35.83), B. megaterium (35.17), B. subtilis (16.5) and B. cereus (43.5). The effect was at B. pumilus, B. megaterium, B. subtilis and B. cereus, but was significantly more for *B. subtilis*. This was also evident in the number of eggs per egg mass and reproduction factor on nematode in B. subtilis treatments. The effect of B. pumilus, B. megaterium, B. subtilis and B. cereus was for with Velum Prime with respect to reduction in galls, average number of egg mass per plant, eggs per egg mass and reproduction factors of nematode. However, B. subtilis was found to result in significantly (P < 0.05) higher reduction in the abovementioned parameters of nematode infection (Table 6).

Discussion

In the past decades, the plant growth-promoting rhizobacteria (PGPR) have gained extensive attention due to their beneficial effects in protecting the host plants against biotic and abiotic stresses (AbdelRazek and Yaseen 2020). They offer sustainable alternative substitutes to agrochemicals in plant protection as well as crop production. In the present research work, bacteria associated with the tomato roots were isolated using 3 media. In all the media, the bacterial densities were found to be higher in uninfected roots, compared to the infected roots and harbored more nematode antagonistic rhizobacteria. The extracellular metabolites of bacteria from uninfected roots of accessions H308, H299 and H266 caused juvenile mortality in the range of 60.67-100% as compared to isolates from nematode infected roots of accessions H195, BSS99, H178/F4 and H88, which caused mortality in the range of 52.33 to 73.67%. Thus, the uninfected roots harbored nematode antagonistic bacterial spp. along with bacterial species that caused less mortality. A higher bacterial diversity resulted in reduced nematode infection in plants. Similar observations were recorded by Abd-El-Khair et al. (2019). On comparing the media used, the soil plate agar, produced the maximum number of colony forming units, compared to soil extraction agar or King's B. Plate count agar or tryptone soya agar as biological substrate for live aerobic bacteria in a sample. It is not a selective medium, but is richer than SEA or King's B, due to the presence of yeast extract (2.5 g/l) and enzymatic digest of casein/tryptone (50 g/l), thus enabling proliferation of more number of bacterial colonies. The rhizobacterial isolates associated with infected and uninfected tomato roots were found significantly different (P < 0.05) with respect to the metabolite toxicity to root-knot juveniles. The 21% bacterial colonies (17 isolates) that caused nemato toxicity in the range of 90 to 100%t and another 15% (12 isolates) that caused juvenile mortality in the range of 80 to 90% were from uninfected tomato roots, indicating their role in protecting the plants against RKN infection. The manipulation of rhizosphere to attain a stable and high diverse microbial structure to prevent the pests and pathogens is highly desirable. However, to know what kind of microbes and the role they play are most important. The microbiome analysis of Zhou et al. (2019) showed that Proteobacteria, Verrucomicrobia, Firmicutes, Nitrospirae and Gemmatimonadetes were more abundant in RKN uninfested soil as compared to infested ones. Moreover, Firmicutes, Pseudomonadales, Enterobacteriales, Rhizobiales, Burkholderiales and Xanthomonadales have been reported as dominant Proteobacteria orders that colonized tomato roots (Tian et al. 2017). Firmicutes to which Bacillus spp. belong are reported as biocontrol agents against

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Treatments	Shoot length (cm)	Increase (%)	Root length (cm)	Increase (%)	Fresh shoot weight (g)	Increase (%)	Fresh root weight (g)	Increase (%)	Biomass (g)	Increase (%)	Root volume (cm ³)	Increase (%)
Water	138.5 ^d		14.7 ^{bc}		196.6 ^c		29.0 ^{de}		225.8 ^c		26.5 ^{de}	
Water + Mi	135.6 ^d		12.1 ^e		170.6 ^d		44.8 ^a		215.0 ^c		24.1 ^e	
B. p	157.8 ^c	13.96	16.7 ^{ab}	13.35	235.3 ^b	19.66	36.0 ^{bc}	24.14	271.3 ^b	20.15	61.0 ^b	129.90
B. p+Mi	163.6 ^{bc}	20.64	11.0 ^e	- 9.09	234.1 ^b	37.21	36.6 ^b	- 18.22	270.0 ^b	25.58	35.1 ^{cde}	45.92
B. m	160.0 ^{bc}	15.52	16.3 ^{ab}	11.20	240.1 ^b	22.12	34.6 ^{bc}	19.54	274.3 ^b	21.48	60.8 ^b	129.21
B. m. + Mi	158.0 ^{bc}	16.38	11.4 ^e	- 5.75	238.3 ^b	39.65	31.83 ^{bcd}	- 29.0	270.3 ^b	25.74	37.3 ^{cd}	54.98
B. s	178.1 ^a	28.64	18.5 ^a	25.68	271.5 ^a	38.05	45.5 ^a	56.90	317.0 ^a	40.37	110.0 ^a	314.57
B. s+Mi	1 76.6 ^a	30.22	14.6 ^{bcd}	20.55	266.8 ^a	56.35	42.1 ^a	- 5.95	309.7 ^a	43.80	45.3 ^c	88.31
B. c	163.3 ^{bc}	17.91	12.5 ^{de}	— 14.93	231.8 ^b	17.88	34.6 ^{bc}	19.54	266.8 ^b	18.15	62.4 ^b	135.18
B. c+Mi	160.3 ^{bc}	18.16	10.6 ^e	- 12.19	239.0 ^b	40.04	24.6 ^e	- 44.98	263.5 ^b	22.56	32.25 ^{de}	33.82
Media (NB)	139.0 ^d	0.36	12.5 ^{cde}	- 15.16	198.6 ^c	0.68	30.6cd	4.02	227.0 ^c	0.74	27.15 ^{de}	2.32
VP	139.3 ^d	0.60	12.0 ^e	- 18.55	197.5 ^c	0.25	34.3 ^{bcd}	9.77	231.6 ^c	2.58	26.53 ^{de}	0.00
VP+Mi	134.3 ^d	- 0.98	10.6 ^e	— 12.47	176.83 ^b	3.61	42.17 ^a	- 5.95	211.5 ^c	- 1.78	29.60 ^{de}	22.82
CD (5%)	5.99		1.18		10.85		3.32		9.47		6.59	
<i>F</i> Value	38.10		32.52		49.53		29.60		66.62		105.74	
Pr (> <i>F</i>)	0.0001		0.0001		0.0001		0.0001		0.0001		0.0001	
Means with th	e same letter are nc	ot significantly diff	ferent									

B. p. Bacillus pumilus, B. m. Bacillus megaterium, B. s. Bacillus subtilis, B. c. Bacillus cereus, Mi, Meloidogyne incognita, V.P. Velum prime (Nematicide)

Devindrappa et al. Egyptian Journal of Biological Pest Control (2022) 32:51



Table 6	Effect of	rhizob	pacterial	isolates	on Me	loido	gyne in	cognita	infect	ing	tomato	cv Pus	sa Ri	ub

No. of galls/plant root	Decrease (%)	No. of J2/cc soil	Decrease (%)	No. of egg mass/plant root	Decrease (%)	No. of eggs/egg mass	Decrease (%)	Reproduction Factor (RF)	Decrease (%)
262.83 ^a		96.33 ^a		162.83ª		259.17 ^a		48.17	
41.50 ^b	- 84.21	42.0 ^c	- 56.40	35.83 ^b	- 77.99	143.33 ^c	- 44.69	21.00	- 56.40
42.83 ^b	- 83.70	47.00 ^{bc}	- 51.21	35.17 ^b	- 78.40	176.67 ^b	- 31.83	23.50	- 51.21
21.17 ^c	- 91.95	31.67 ^d	- 67.13	16.50 ^c	- 89.87	86.50 ^d	- 66.62	15.83	- 67.13
47.67 ^b	- 81.86	52.67 ^d	- 45.33	43.50 ^b	- 73.29	179.67 ^b	- 30.87	26.33	- 45.33
44.33 ^b	- 83.13	48.50 ^{bc}	- 49.65	45.67 ^b	- 71.95	173.33 ^b	- 33.12	24.25	- 49.65
9.11		5.62		8.77		14.01			
808.39		137.03		288.42		169.69			
0.0001		0.0001		0.0001		0.0001			

Means with the same letter are not significantly different

B. p, Bacillus pumilus, B. m, Bacillus megaterium, B. s, Bacillussubtilis, B. c, Bacillus cereus, Mi, Meloidogyne incognita, VP, Velum prime (Nematicide)

soil-borne pathogens and plant parasitic nematodes with the ability to produce several lytic enzymes and antimicrobial secondary metabolites (Akinrinlola et al. 2018). In the present study, the selected isolates of *B. pumilus, B. megaterium, B. subtilis* and *B. cereus* with highly nematicidal and multiple plant growth promotion traits that play a major role in plant root architecture and nutrients acquisition. These microorganisms were reported to alter the auxin pathways in the host plant, leading to faster completion of the pre-germination metabolic activities to make seed ready for radicle emergence and improved seed germination and seedling vigor index as compared to untreated seeds (Sukumar et al. 2013). Catalase activity is an important trait of bacteria as it helps the bacteria, to protect itself against hydrogen peroxide that is harmful to bacteria and plant roots. Therefore, PGPR with catalase activity survive better in the rhizosphere and promote plant growth indirectly. Due to their mycolytic activities, chitinase-producing bacteria prevents the proliferation of phytopathogens and can be detrimental to nematode eggs. The ammonia production by the rhizobacteria accumulates and supplies nitrogen to their host plant and promotes root and shoot elongation and their biomass (Fahsi et al. 2021). The level of HCN produced by the rhizobacteria in vitro does not correlate with the observed biocontrol effects, rather helps in phosphate solubilization. The indirect increase of nutrient availability is beneficial for the rhizobacteria and their plant hosts (Tomaz and Lapanje 2016).

Conclusions

Plant roots were associated with *B. pumilus, B. megaterium, B. subtilis* and *B. cereus* that were associated with nematode free plants in nematode infested soil. These isolates can be utilized in nematode pest management in infested polyhouses, especially where tomato is cultivated. Their efficacy on other polyhouse crops like cucurbits and capsicums and floral crops like Gerberas needs to be ascertained.

Abbreviations

RKN: Root-knot nematode; PGPR: Plant growth-promoting rhizobacteria; J2: Juveniles; IAA: Indole acetic acid; 16s RNA: Ribosomal nucleic acid; DNA: Deoxyribonucleic acid; Spp.: Species; SPCA: Soil plate count agar; SEA: Soil extract agar; NB: Nutrient broth; CFF: Cell-free culture filtrate; HCN: Hydrogen cyanide.

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Author contributions

AK and DM both conceived and designed the experiment, DM has collected the data, analyzed the data and wrote the paper, AK corrected the paper MG, and BG assisted in experiments conducted. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Page 11 of 11

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