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Biocontrol potential of bacterial isolates from vermicompost and earthworm against the root-knot nematode *Meloidogyne javanica* infecting tomato plants

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Abstract

Background: Root-knot nematodes (*Meloidogyne* spp.) are the most destructive agricultural pests, which parasitize thousands of different plant species in the world. Using antagonistic bacteria can be a potential alternative to hazardous chemical nematicides. This study was conducted to evaluate the biocontrol activities of the bacteria isolated from vermicompost and earthworm against *M. javanica* in infected tomato plants.

Results: Seventeen bacteria were isolated from vermicompost and earthworm. Their antagonistic effects were tested against the root-knot nematode *M. javanica* in laboratory and in glasshouse experiments. In the preliminary screening test, 8 bacterial isolates significantly caused more than 50% decrease in reproduction factor (Rf) of the nematode on tomato plants. Six isolates with more than 60% reduction in the nematode Rf were selected and identified as follows: *Lysinibacillus fusiformis* C1, *Bacillus megaterium* C3, *B. safensis* VW3, *Pseudomonas resinovorans* VW4, *Lysinibacillus* sp. VW6, and *Sphingobacterium daejeonense* LV1 by 16S rRNA gene sequencing. The isolates *B. megaterium* C3, *B. safensis* VW3, *P. resinovorans* VW4, and *L. fusiformis* C1 inhibited the nematode egg hatching by 20–28%, and *Lysinibacillus* sp. VW6 and *L. fusiformis* C1 caused 15 and 20% mortality of the second-stage juveniles in vitro. In a glasshouse, the 6 bacterial isolates reduced the nematode Rf by 47–66%, and *P. resinovorans* VW4 was the most effective isolate. However, *B. safensis* VW3, *B. megaterium* C3, and *L. fusiformis* C1 had the best effect on plant growth.

Conclusions: Most of the bacteria isolated from earthworm or vermicompost had nematicidal properties. This study provided empirical evidence of the nematicidal potential of isolates *Lysinibacillus fusiformis* C1, *Pseudomonas resinovorans* VW4, and *Sphingobacterium daejeonense* LV1 and the antagonistic activities of *Bacillus megaterium* C3 and *B. safensis* VW3 against *Meloidogyne javanica*.

Keywords: *Bacillus megaterium*, *Bacillus safensis*, Biocontrol, Earthworm, *Lysinibacillus fusiformis*, *Pseudomonas resinovorans*, *Sphingobacterium daejeonense*

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Background

Root-knot nematodes (*Meloidogyne* spp.) are the most destructive agricultural pests, which parasitize many plant species of different groups around the world. They are the main subject of extensive research, including studies on biology, plant-nematode interaction, and especially control approaches (Moens et al. 2009). Many efforts have been made to find appropriate alternatives to environmentally harmful nematicides in the management of plant-parasitic nematodes (Oka et al. 2000).

Many parasitic and non-parasitic species of rhizobacteria are able to decrease the nematode population (Siddiqui and Mahmood 1999). Bacteria affect plant-parasitic nematodes directly or indirectly via producing secondary metabolites and toxins or inducing systemic resistance in the plant (Mhatre et al. 2019).

The plant growth-promoting genera *Azotobacter*, *Bacillus*, *Serratia*, and *Pseudomonas* not only improve the plant growth but also contribute to nematode management (Wani 2015). *Bacillus pumilus*, *Paenibacillus castaneae*, and *Mycobacterium immunogenum* affected *M. incognita* in a greenhouse experiment by reducing the number of eggs and nematode galls and increasing tomato plant growth (Cetintas et al. 2018). Most detrimental rhizobacteria affect plant-parasitic nematodes via different mechanisms (Siddiqui and Mahmood 1999). Antagonistic bacteria kill nematodes by producing antibiotics, enzymes including proteases and chitinases, toxins, and volatile compounds (Marin-Bruzos and Grayston 2019). In addition, plant growth-promoting rhizobacteria (PGPR) induced systemic resistance by increasing the activity of the defense gene, which caused the decrease in the level of root-knot nematode infection in tomato plants (Vigila et al. 2019). These bacteria have been extracted from various substrates including manure, compost, and soil.

Vermicompost and earthworms are rich sources of beneficial bacteria (Pathma and Sakthivel 2013). Application of earthworm in the soil increases population densities of the fluorescent pseudomonads and actinomycetes (Elmer 2009). Metagenomic analyses of intestinal bacterial flora of earthworms showed the presence of numerous bacteria species, generally belonging to *Proteobacteria* (Singh et al. 2015). Some of the bacteria frequently found in vermicompost include *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Nitrobacter*, *Paenibacillus*, *Pseudomonas*, and *Spiroplasma* (Pathma and Sakthivel 2013). Worm casts are a rich source of antagonistic bacteria that can suppress plant pathogens including parasitic nematodes (Pathma and Sakthivel 2012).

Some reports showed that vermicompost had the ability to suppress several groups of plant-parasitic nematodes (Renčo and Kováčik 2015; Xiao et al. 2016). To

the best of our knowledge, there is no report on the nematicidal activity of the bacteria, extracted from vermicompost and earthworm. Therefore, this study was conducted to evaluate the effects of bacteria, which isolated from vermicompost and earthworm on the root-knot nematode *Meloidogyne javanica* in vitro and in the glasshouse.

Methods

In the present study, bacteria were isolated from the coelomic fluid of earthworm, vermicompost leachate, and vermiwash. In a preliminary experiment, the effect of the bacterial isolates on *M. javanica* on tomato plants in a glasshouse was investigated. Then, the effects of the most effective isolates on egg hatching, mortality of the second-stage juveniles (J2s) of the nematode in laboratory, and the nematode activity and growth indices of infected tomato plants in the glasshouse were investigated.

Collection and inoculum of *Meloidogyne javanica*

Severely cucumber galled roots with *Meloidogyne* sp. were collected from an infested greenhouse in Fars province, Iran. By using the single egg mass technique, the nematode was reared on tomato (*Lycopersicon esculentum* Mill. cv. Early Urbana) roots. The nematode was identified as *M. javanica* using specific primers of *M. javanica*, *M. incognita*, and *M. arenaria* (Dong et al. 2001). The nematode eggs were extracted from the galled roots using sodium hypochlorite (Hussey and Barker 1973). After washing with tap water, the infected roots were cut into 2–3-cm-long pieces and mixed with 0.5% NaOCl in a blender. The roots were chopped in the blender for 30 s at low speed, followed by passing through 20-, 200-, and 500-mesh/in. sieves. Eggs on the 500-mesh sieve were gently washed by water to free them from NaOCl and collected into a Petri dish.

Isolation of bacteria

Vermicompost of animal manure was prepared during 3 months of summer. Liquid vermicompost, vermiwash, and the coelomic fluid of the earthworm *Eisenia fetida* were applied to isolate the bacteria (Rostami et al. 2014). For preparing the liquid vermicompost, a vermicompost rucked bag (100 g) was immersed into the water bucket (1 l) for 2 days at room temperature. Then, the water bucket was aerated strongly by using an air pump for 1 day.

Vermiwash was obtained by adding 50 g earthworms to 500 ml of warm water and kept at room temperature for 30 min. Then, it was stirred by a glass rod for 3 min and centrifuged at 3000 rpm for 10 min to separate and sediment insoluble portion (Rostami et al. 2014).

To obtain the coelomic fluid, 50 g earthworms were placed into a Petri dish containing a sterile saline

solution (0.9%) and then subjected to electric shock every 3 s, using a 9-V battery. The obtained coelomic fluid was used to isolate bacteria (Rostami et al. 2014).

To isolate bacteria, 5 µl of each of the 3 liquid sources was cultured on nutrient agar (NA) and incubated for 48 h at 28 °C.

Preliminary evaluation of the bacterial isolates against *Meloidogyne javanica*

The inhibitory effects of 17 bacteria isolated from vermicompost leachate, vermiwash, and the earthworm coelomic fluid on *M. javanica* activities were studied in a glasshouse. The seeds of tomato (cv. Early Urbana) were sown in 19-cm diameter plastic pots containing 3 kg pasteurized mixed soil (field soil and river sand, 1:2); 40 mg P (kg soil)⁻¹ equivalent to 250 mg triple super phosphate and 60 mg N (kg soil)⁻¹ equivalent to 360 mg potassium nitrate were applied to the soil at sowing. Plants at the four-leaf stage were inoculated with 20 ml of bacteria suspensions (10⁸ CFU/ml) per pot. Three days later, the tomato seedlings were inoculated with 6000 eggs of the root-knot nematode by adding the eggs to small holes made in the soil around the roots. The pots were placed on a glasshouse bench at 25–30 °C in a completely randomized design with 4 replicates. The pots were observed daily and watered as needed. Ninety days after nematode inoculation, the plants were harvested and their fresh root weights were measured. Then, the nematode second-stage juveniles (J2s) in a 100-g mixed soil sample of each pot were extracted by the Whitehead and Hemming tray method (Whitehead and Hemming 1965). In addition, the number of galls and egg masses in 1 g of roots of each plant were counted after staining with fuchsin acid. Moreover, the eggs in 1 g of root were extracted and counted. Then, the final population (Pf) and the reproduction factor (Rf) of *M. javanica* were calculated ($Rf = Pf/Pi$).

After the preliminary experiment, the effects of the 6 selected bacterial isolates were investigated to evaluate their nematocidal ability against *M. javanica*. The conditions of the glasshouse experiment were the same as described in the preliminary experiment.

In vitro evaluation of the nematocidal ability of the selected bacterial isolates

Bacteria were cultured on NA and incubated for 48 h at 28 °C in the incubator. To study their effects on the nematode egg hatching and juvenile mortality, about 100 eggs and 100 J2s per 1 ml sterile water were added in separate 6-cm Petri dishes. Then, 5 ml of bacteria suspension (10⁸ CFU/ml) were added. Distilled water was chosen as the control. After 48 h, the dead J2s were counted, and after 72 h, the unhatched eggs were

counted (Cayrol et al. 1989). The experiment was carried out as a completely randomized design with 3 replicates.

Statistical analysis

Data of laboratory and glasshouse experiments were subjected to analysis of variance by the SAS 9.1 software. The comparison of means was done with Duncan's multiple range test ($P < 0.05$) (Duncan 1955).

Identification of bacterial isolates

The Gram reactions of bacterial isolates were determined by the 3% KOH test (Schaad et al. 2001). The isolates were cultured on King's B medium (KB) to detect fluorescein production (King et al. 1954), and their tolerance to NaCl was tested (Caton et al. 2004).

Bacterial DNA was extracted using the Expin™ Combo GP (GeneAll®, Tic Tech Centre, Singapore) DNA extraction kit, following the protocol of the manufacturer. The quality and quantity of the DNAs were spectrophotometrically evaluated and adjusted to 50 ng µl⁻¹, using the Nanodrop ND-100 (Nanodrop Technologies, Waltham, MA, USA).

The complete 16S rDNA was amplified using universal bacterial primers 27F (5'-AGAGTTTGATCCTGGC TCAG-3') and 1492R (5'-GGTACCTTGTACGACT T-3'). The reaction mixture was 1 µl of DNA (50/µl), 1 µl each of forward and reverse primers (10 µM), 10 µl AmpliQon®Taq DNA Polymerase Master Mix Red (Ampliqon A/S, Odense, Denmark), and 7 µl of double-distilled water. The PCR was performed (Kumar et al. 2014). Then, the PCR products were subjected to sequencing (Microsynth Company, Switzerland).

The obtained sequences were analyzed by BLASTn (NCBI (<http://blast.ncbi.nlm.nih.gov>)). The sequences of related species and genera were obtained from the GenBank database, and the phylogenetic analysis was carried out with MEGA version 7 (Kumar et al. 2016). The sequences were lined up by Clustal W (Larkin et al. 2007) and analyzed using the maximum likelihood method to make a phylogenetic tree showing the relationships among isolates with percentage bootstrap values based on 1000 replicates (Saitou 1988).

Results

Preliminary evaluation of the bacterial isolates against *Meloidogyne javanica*

The results of preliminary screening of the 17 bacterial isolates from vermicompost or earthworm showed that in all treatments, the number of galls per gram of infected tomato roots was significantly lower than the untreated control. Except for one, the other 16 isolates reduced the Rf of the nematode by 11.6 to 81.6%. However, 8 out of 16 isolates significantly reduced the nematode Rf from 54.8 to 81.6%, while 6 of them with

more than 60% reduction were selected for further in vitro and glasshouse experiments (Table 1).

In vitro evaluation of the nematicidal ability of the selected bacterial isolates

The selected bacterial isolates affected egg hatching and J2 mortality of *M. javanica* differently. The egg hatching of the nematode in the presence of *Lysinibacillus* sp. (W6) and *S. daejeonense* LV1 was statistically similar to the control (water). However, *B. megaterium* C3, *P. resinovorans* VW4, *B. safensis* VW3, and *L. fusiformis* C1 significantly inhibited egg hatching of the nematode by 20 to 28%. The effects of the bacterial isolates on J2 mortality were somewhat different from their effects on egg hatching. The greatest death of J2s happened in the presence of *Lysinibacillus* sp. (W6) and *L. fusiformis* C1.

The effects of *B. megaterium* C3 and *P. resinovorans* VW4 on J2s were statistically similar to the control. The isolates *Lysinibacillus* sp. (W6), *L. fusiformis* C1, *B. safensis* VW3, and *S. daejeonense* LV1 caused 10 to 20% J2 mortality (Fig. 1).

Evaluation of the effects of selected bacteria on nematode indices and plant growth parameters of tomato infected with *Meloidogyne javanica*

Plant growth indices

All selected isolates, except *P. resinovorans* VW4, significantly ($P \leq 0.05$) improved shoot fresh and dry weights of the uninfected tomato plants (Table 2). *B. safensis* VW3, *B. megaterium* C3, and *L. fusiformis* C1 significantly increased both shoot fresh and dry weights of the infected tomato plants. In addition, *S. daejeonense* LV1

Table 1 Screening the effects of 17 bacterial isolates from vermicompost and earthworm on the nematode indices of *Meloidogyne javanica* in the roots of tomato plants in a glasshouse

Bacterial isolates ^a	Galls/g root	Egg masses/g root	Eggs/g root	J2s/pot soil	Root fresh weight (g)	Final population (Pf)	Reproduction factor (Rf)	Rf reduction (%) ^b
C1	183 b	100 d–g	4238 cd	5700 g	6.00 b–f	33,031 cd	5.5 cd	66.0
C2	158 bc	122 c–g	7744 b–d	18,000 a–d	8.12 ab	82,417 ab	13.7 ab	15.3
C3	151 bc	67 gf	2663 d	11,100 b–g	5.67 b–f	26,424 b–d	4.4 b–d	72.8
C6	123 bc	184 b–d	7200 b–d	22,500 a	5.95 b–f	65,865 a–c	10.9 a–c	32.3
C8	107 bc	164.5 b–e	12,338 ab	21,450 ab	6.00 b–f	97,487 a	16.2 a	0.0
C9	148 bc	149 c–f	9225 bc	13,500 a–g	8.12 ab	86,058 a	14.3 a	11.6
LV1	128 bc	50 g	3300 d	9975 d–g	5.02 c–f	27,930 cd	4.6 cd	71.3
LV2	110 bc	200 a–c	9375 bc	17,100 a–f	5.15 b–f	58,271 a–c	9.7 a–c	40.1
LV3	131 bc	140 c–f	7650 b–d	17,700 a–e	7.95 a–c	81,350 ab	13.5 ab	16.4
LV5	113 bc	172.5 b–e	6694 cd	15,900 a–g	6.80 a–d	62,962 a–c	10.4 a–c	35.3
LV6	158 bc	154 b–e	6038 cd	15,750 a–g	4.40 d–f	41,249 cd	6.8 cd	57.3
LV8	131 bc	235 ab	8275 b–d	16,275 a–g	6.62 a–e	59,056 a–c	9.8 a–c	39.3
VW2	175 bc	205 a–c	6435 cd	19,200 a–d	3.65 ef	43,931 b–d	7.3 b–d	54.8
VW3	122 bc	92 e–g	5025 cd	10,275 d–g	4.90 d–f	36,393 cd	6.0 cd	62.6
VW4	95 c	41 g	3225 d	6900 e–g	5.77 b–f	25,751 cd	4.2 cd	73.5
VW5	110 bc	196.5 a–c	6875 b–d	21,150 a–c	9.15 a	85,636 a	14.2 a	12.0
VW6	161 bc	121 e–g	3075 d	6390 f–g	3.55 f	17,895 d	2.9 d	81.6
Water	328 a	275 a	16,650 a	20,475 a–d	4.35 d–f	97,404 a	16.2 a	–

Data are the means of 4 replicates. Values in the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$), according to Duncan's multiple range test

^aBacterial isolates from the coelomic fluid of earthworm (C), Liquid vermicompost (LV), and Vermiwash (VW)

^bPercentage of the nematode reproduction factor (Rf) reduction compared to the control

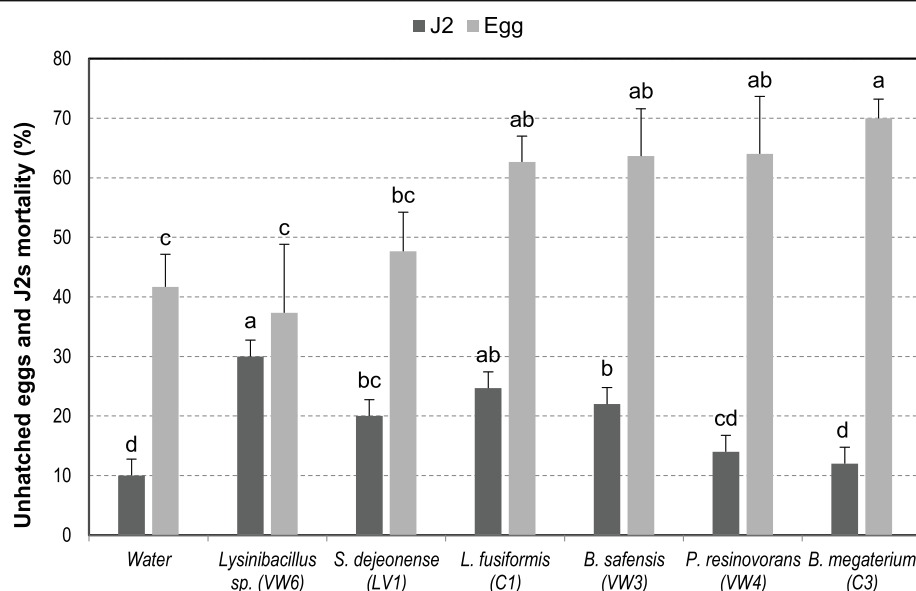


Fig. 1 Effect of bacteria isolates on egg hatching and mortality of the second-stage juveniles (J2s) of *Meloidogyne javanica* in vitro. Values in the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$), according to Duncan's multiple range test. Bars indicate the standard error of the means ($n = 3$)

improved these indices, but its effect was less than the others. *P. resinovorans* VW4 only increased the shoot fresh weight of infected plants.

Nematode indices

The six bacterial isolates significantly decreased the number of galls, egg masses, and eggs per gram of root, and final population of the root-knot nematode (Table 3). The isolate *P. resinovorans* VW4 had the greatest effect on reducing the number of galls, egg masses, and eggs in the root system, and the nematode Rf. The selected bacterial isolates reduced the nematode Rf by 47–66%.

Molecular identification of bacterial isolates

Identification of isolates was done by comparison of their 16S rDNA sequences with those deposited in the GenBank database. The 6 selected isolates were identified as *Bacillus safensis* VW3, *Pseudomonas resinovorans* VW4, *Lysinibacillus* sp. VW6, *L. fusiformis* C1, *B. megaterium* C3, and *Sphingobacterium daejeonense* LV1. The sequences of their 16s rDNA had been deposited in the GenBank (Table 4). The phylogenetic relationship among the identified bacterial isolates and the closely related species and genera has been shown in (Figs. 2 and 3).

P. resinovorans VW4 and *S. daejeonense* LV1 are gram-negative, but *B. safensis* VW3, *B. megaterium* C3,

Table 2 Effect of bacterial isolates on the growth of tomato plants infected by *Meloidogyne javanica*

Treatments	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g), with nematode
	With nematode	Without nematode	With nematode	Without nematode	
<i>Bacillus megaterium</i> C3	29 a–c	29 a–c	3.47 a–c	3.90 a–d	7.17 a
<i>Bacillus safensis</i> VW3	35 a	35 a	4.72 ab	5 a	4.27 a
<i>Lysinibacillus fusiformis</i> C1	25 a–d	30 ab	3.77 a–d	3.85 a–d	4.10 a
<i>Lysinibacillus</i> sp. VW6	17 c–e	32 a	2.25 d–f	3.50 a–c	4.05 a
<i>Pseudomonas resinovorans</i> VW4	18 b–d	27 a–d	2.52 d–f	3.02 de	5.47 a
<i>Sphingobacterium daejeonense</i> LV1	23 a–d	35 a	1.90 e	4.82 a	4.62 a
Water	8.65 e	13 d	1.27 f	1.75 e	4.58 a

Data are the means of 4 replicates. Values in the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$), according to Duncan's multiple range test

Table 3 Effects of six selected bacterial isolates on nematode indices of *Meloidogyne javanica* on tomato roots in a glasshouse

Treatments	Galls/g root	Egg masses/g root	Eggs/g root	J2s/pot soil	Final population (Pf)	Reproduction factor (Rf)	Rf reduction (%) ^a
<i>Bacillus megaterium</i> C3	110 bc	127 b	4475 bc	8850 b	38,260 b	6.3 b	47.2
<i>Bacillus safensis</i> VW3	119 bc	117 bc	5825 b	8100 b	32,957 b	5.4 b	54.5
<i>Lysinibacillus fusiformis</i> C1	141 b	67 cd	7875 bc	4500 b	36,798 b	6.1 b	49.2
<i>Lysinibacillus</i> sp. VW6	123 bc	133 b	5525 bc	5850 b	28,730 b	4.7 b	60.3
<i>Pseudomonas resinovorans</i> VW4	72 c	48 d	3738 c	6300 b	24,615 b	4.1 b	66.0
<i>Sphingobacterium daejeonense</i> LV1	122 bc	50 d	5529 bc	6320 b	33,765 b	5.6 b	53.4
Water	251 a	216 a	11,400 a	15,900 a	72,526 a	12.0 a	–

Data are the means of 4 replicates. Values in the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$), according to Duncan's multiple range test

^aPercentage of the nematode reproduction factor reduction compared to the control

Lysinibacillus sp. VW6, and *L. fusiformis* C1 are gram-positive. None of them could produce fluorescein on King's B medium. While all isolates grow on medium with 2% NaCl, but only *B. safensis* VW3 tolerate 10% NaCl (Table 4).

Discussion

Antagonistic bacteria against the *M. javanica* were isolated from liquid vermicompost, coelomic fluid of earthworm, and vermiwash, which were chosen as the rich source of bacteria. The most effective isolates belonging to the genera *Bacillus*, *Lysinibacillus*, *Pseudomonas*, and *Sphingobacterium*. The nematicidal activity and their stimulating effects on plant growth of some isolates of these genera have already been studied and shown. However, the effects of some isolates of the present study have not been previously demonstrated.

The genus *Bacillus* includes many species, which mostly isolated from soil and exhibit plant-promoting traits. The controlling effects of several species including *B. cereus*, *B. subtilis*, and *B. megaterium* on plant-parasitic nematodes have been shown in many studies

(Saikia et al. 2013), and Engelbrecht et al. (2018) summarized their usefulness as natural enemies of root-knot nematodes, their nematicidal activities, and their mode of action as biocontrol agents. In the present study, the isolates *B. megaterium* C3 and *B. safensis* VW3 improved the growth parameters of the nematode infected and healthy tomato plants. Moreover, they reduced the nematode Rf by 72.8 and 62.6% in the first screening and 47.2 and 54.5% in the second selected isolates glasshouse experiments. The extent of this effect changed in the second experiment, but significantly, both *Bacillus* isolates reduced the nematode indices. The second experiment can confirm that by repeating the experiment, these bacteria were still effective in controlling nematode damage.

The effect of *B. safensis* VW3 on the plant growth parameter and the nematode indices was slightly better than *B. megaterium* C3. On the other hand, both species significantly inhibited the nematode egg hatching, but only *B. safensis* VW3 caused J2 mortality.

Strains of *B. safensis* had the ability to produce amylase, chitinase, keratinase, lipase, protease, and some

Table 4 The bacterial isolates from liquid vermicompost, vermiwash, or the coelomic fluid of earthworm, and their accession numbers of their 16S rDNA sequences deposited in the GenBank database

Bacterial isolates	Sources	Gram reaction	Fluorescence on King's medium B	2% NaCl tolerance	5% NaCl tolerance	7% NaCl tolerance	10% NaCl tolerance	Accession no.
<i>Bacillus megaterium</i> C3	Coelomic fluid	+	–	+	+	+	–	MN560028
<i>Bacillus safensis</i> VW3	Vermiwash	+	–	+	+	+	+	MN560025
<i>Lysinibacillus fusiformis</i> C1	Coelomic fluid	+	–	+	+	+	–	MN560027
<i>Lysinibacillus</i> sp. VW6	Vermiwash	+	–	+	+	–	–	MN560026
<i>Pseudomonas resinovorans</i> VW4	Vermiwash	–	–	+	–	–	–	MN559969
<i>Sphingobacterium daejeonense</i> LV1	Liquid vermicompost	–	–	+	–	–	–	MN559970

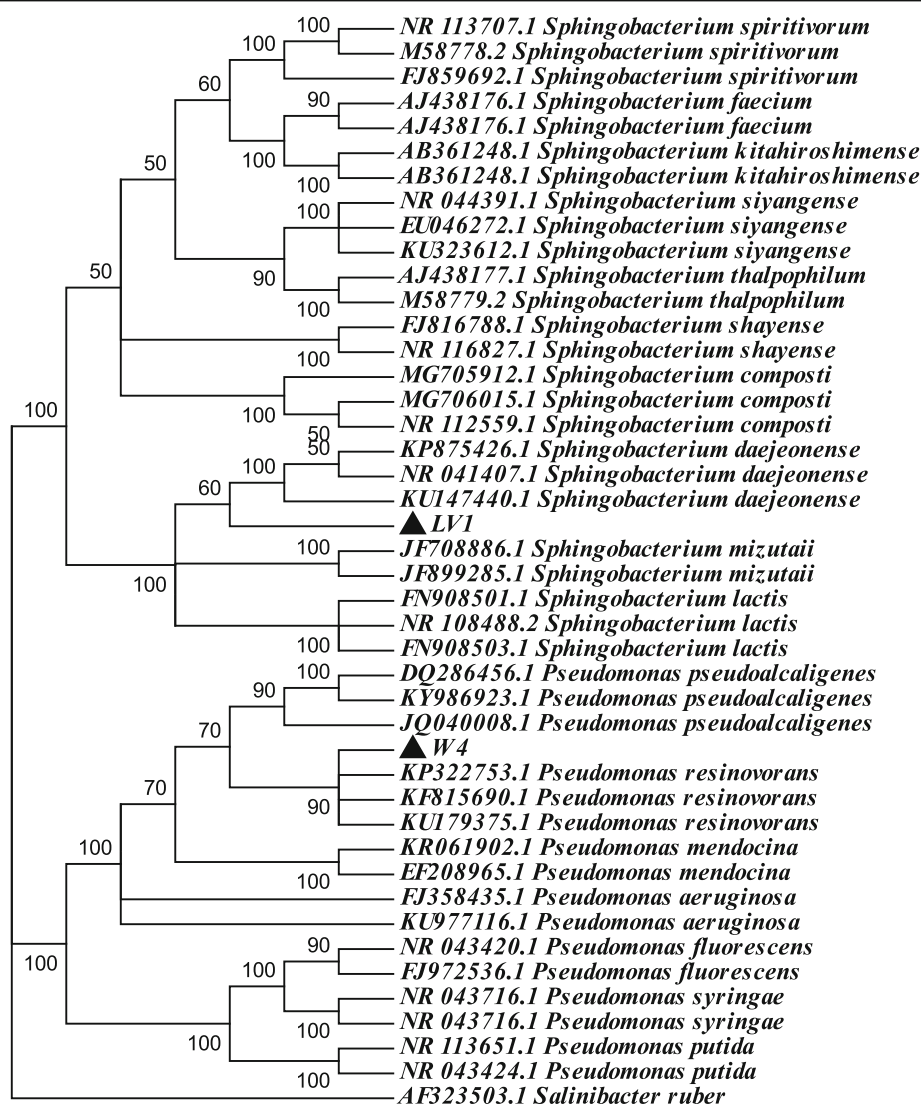


Fig. 2 Phylogenetic relationships of *Pseudomonas resinovorans* (W4) and *Sphingobacterium daejeonense* (LV1) based on 16S rDNA sequences (maximum likelihood method). Numbers on the branches indicate bootstrap values. *Salinibacter ruber* was used as a root

other enzymes (Lateef et al. 2015a). *B. safensis* can promote plant growth (Lateef et al. 2015b), and this feature has been shown in several studies. *B. safensis* R173 and *B. megaterium* R181 along with two other strains, which isolated from the wheat rhizosphere, were the most efficient strains on corn growth in greenhouse pot test (Akinrinlola et al. 2018). In two simultaneous studies, the nematicidal activity of *B. safensis* has been demonstrated. The results of an in vitro screening of plant growth-promoting rhizobacteria (PGPR) indicated that three strains Bsa25, Bsa26, and Bsa27 of *B. safensis* and 12 other species of *Bacillus* caused greater than 50% *H. glycines* J2 mortality. In addition, the strain *B. safensis* Bsa27 decreased the number of *H. glycines* cysts at 60

days after planting in a field experiment (Xiang et al. 2017a). In another study, Xiang et al. (2017b) reported that strains of *B. safensis*, such as Bsa26 caused 53.7 to 100%, mortality of *M. incognita* J2s, and *B. safensis* along with other *Bacillus* species showed antagonistic activity against *M. incognita*. As shown in this study, *B. safensis* isolate was able to reduce the population of root-knot nematode.

B. megaterium is an aerobic and large-cell bacterium, which was found in soil and several plant tissues as an endophyte. Some of its isolates involved in chitin degradation, nitrogen fixation, or solubilization of insoluble phosphates (Logan and De Vos 2015). An endophytic isolate of *B. megaterium*, which was found in root

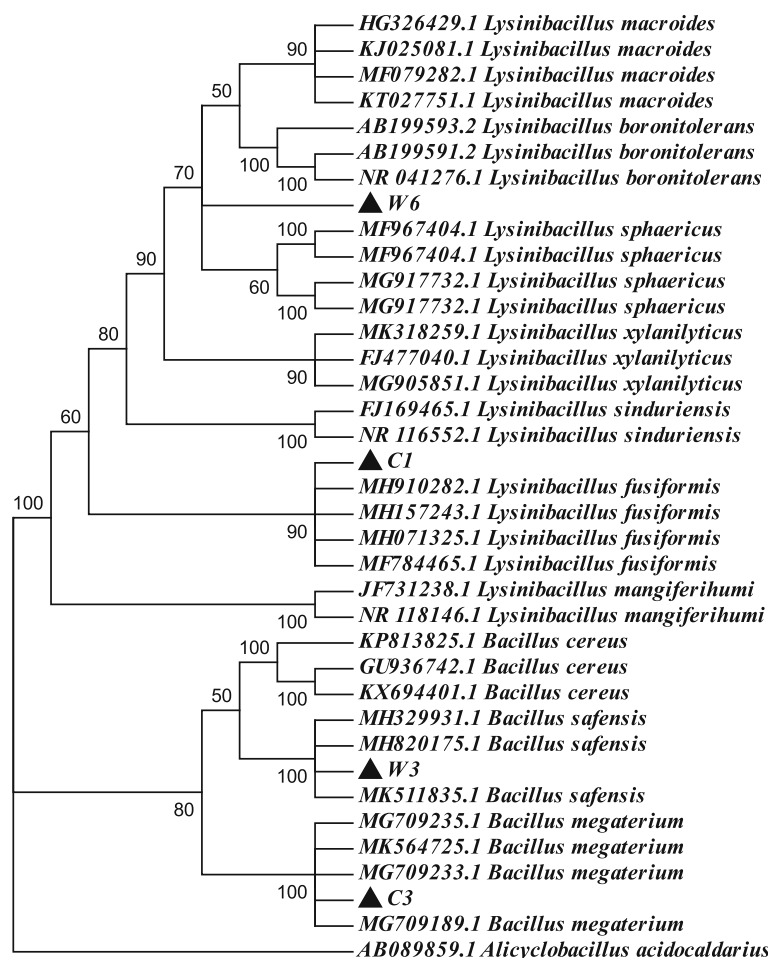


Fig. 3 Phylogenetic relationships of *Bacillus* spp. (*B. megaterium* C3 and *B. safensis* VW3) and *Lysinibacillus* spp. (*L. fusiformis* C1 and *Lysinibacillus* sp. VW6) based on 16S rDNA sequences (maximum likelihood method). Numbers on the branches indicate bootstrap values. *Alicyclobacillus acidocaldarius* was used as a root

nodules of *Medicago polymorpha*, was able to produce indole acetic acid (IAA) (Chinnaswamy et al. 2018). In addition, an isolate of *B. megaterium* reduced migration of *M. graminicola* to the root zone of rice plants and its root penetration (Padgham and Sikora 2007). Huang et al. (2010) showed that the strain *B. megaterium* YMF3.25 reduced egg hatching and infection of *M. incognita* by producing nematicidal volatiles. The results of an experiment indicated that five isolates of *Bacillus* out of 34 strains of endophytic bacteria, which isolated from healthy roots of black pepper, caused 100% mortality of *Meloidogyne* sp. J2s in vitro. Of these selective strains, *B. megaterium* DS9 significantly reduced the nematode populations in soil and roots of infected pepper plants by 81.86% and 73.11%, respectively, in a greenhouse (Tran et al. 2019).

The effects of *B. safensis* VW3 and *B. megaterium* C3 on egg hatching could be related to their ability to produce chitinase. The eggshell is the only

structure of the nematode that contains chitin. Both species are PGPR and can improve plant growth and are nematicidal. It has been shown that there was a positive correlation between the chitinase production and nematicidal abilities of the bacterial strains and their effect on the plant growth parameters (Abdel-Salam et al. 2018).

In the present study, 2 isolates of *Lysinibacillus* showed nematicidal activity. *L. fusiformis* C1 isolated from coelomic fluid of earthworm and *Lysinibacillus* sp. VW6 from vermiwash. *L. fusiformis* C1 caused 66 and 49.2%, and *Lysinibacillus* sp. VW6 81.6% and 60.3% reduction the nematode Rf, respectively in the first screening and second selected isolates glasshouse experiments. Both isolates caused the greatest death rate of *M. javanica* J2s in vitro by 15 and 20%, respectively, but only *L. fusiformis* C1 significantly inhibited the nematode egg hatching by 21% and improved the infected tomato plant growth parameters.

Several species of the genus *Lysinibacillus* reported having nematocidal activity. *L. mangiferae*, which was separated from the soil around the roots of mango, produced nematocidal volatile compounds against *M. incognita* (Yang et al. 2012). It was reported that *L. macroides* caused 64.8% mortality of *M. incognita* J2s in a laboratory trial (Xiang et al. 2017b). As far as we know, the nematocidal activity of *L. fusiformis* has not been reported yet. Singh et al. (2013) demonstrated that the strain *L. fusiformis* B-CM18, which was collected from chickpea rhizosphere and has the ability to produce chitinase, possesses antifungal activity against different fungal pathogens. *L. fusiformis* C1, which inhibited egg hatching of *M. javanica*, is likely to produce chitinase.

Another bacterium with inhibitory effect on *M. javanica* activities in the present study was *Pseudomonas resinovorans* VW4, which was isolated from vermiwash. It caused the greatest reduction in galls and egg masses number and reduced nematode Rf by 73.5 and 66.0% in the first screening and second selected isolates experiments, respectively. Moreover, it inhibited 22% egg hatch of *M. javanica*, but did not affect J2 mortality and the growth parameters of infected tomato plants.

Many *Pseudomonas* species promote plant growth, induce resistance, and have the ability to protect the plant against pathogens (Preston 2004; Osman et al. 2011). *P. aeruginosa* is a plant growth-promoting rhizobacterium, which its nematocidal effects on root-knot nematodes were reported. Siddiqui and Ehteshamul-Haque (2001) reported that *P. aeruginosa* IE-6S⁺ reduced the population of *M. javanica* in soil and infected tomato roots. This isolate was able to produce hydrogen cyanide (HCN). Two other isolates of this bacterium, Pa8 and Pa9, reduced egg hatching of *M. incognita*, increased the tomato plant growth, decreased the galls number and nematode reproduction in a glasshouse test, and produced a great amount of HCN and IAA (Singh and Siddiqui 2010). The nematocidal effects of the Antarctic strain *P. putida* 1A00316 on *M. incognita*, showed in the pot and in vitro experiments, indicated that this strain could increase the activities of phenylalanine ammonia lyase, polyphenol oxidase, and peroxidase as defense enzymes, and induce systematic resistance in tomato plants (Tang et al. 2014). In addition, it was shown that 7 volatile compounds of strain 1A00316 had nematocidal activity against *M. incognita* J2s and inhibited its egg hatching when used directly or as a fumigant (Zhai et al. 2018). Although the nematocidal potential of several species of *Pseudomonas* has been demonstrated, there is no report recorded concerning the nematocidal activity of *P. resinovorans*.

In the present study, *S. daejeonense* LV1, which was isolated from liquid vermicompost, showed the nematocidal effect on *M. javanica* and caused 71.3 and 53.4%

reduction in the nematode Rf respectively in the first and second glasshouse experiments. It caused *M. javanica* J2 mortality and improved the growth parameters of healthy and infected tomato plants but did not affect the egg hatching. Although there are no reports concerning the nematocidal activity of *S. daejeonense*, the effect of other species on plant-parasitic nematodes has been demonstrated. *S. nematocida*, the endophyte bacterium, which has been isolated from the fresh leaf of *Nicotiana tabacum* in China (Liu et al. 2012), caused 100% mortality of juveniles and inhibited 100% egg hatching of *M. incognita* (Xi et al. 2013). In addition, it was shown that *Sphingobacterium* sp. CIGBTb produced chitinase and had nematocidal effects on root-knot nematodes (Sánchez Ortiz et al. 2018).

Conclusions

Most of the bacteria isolated from earthworm or vermicompost had nematocidal activities. The bacterial isolates *Bacillus megaterium* C3, *B. safensis* VW3, *Lysinibacillus* sp. VW6, *L. fusiformis* C1, *Pseudomonas resinovorans* VW4, and *Sphingobacterium daejeonense* LV1 showed biocontrol potentials against the root-knot nematode *M. javanica* in the infected tomato plant. Moreover, the isolates *B. megaterium* C3, *B. safensis* VW3, and *L. fusiformis* C1 improved the growth parameters of tomato plants. The nematocidal activities of isolates *L. fusiformis* C1, *P. resinovorans* VW4, and *S. daejeonense* LV1 and the antagonistic activities of *B. megaterium* C3 and *B. safensis* VW3 against *M. javanica* are first documented in this study. Further studies to investigate the nematocidal effects of the bacteria under field conditions and their mode of actions are needed.

Abbreviations

HCN: Hydrogen cyanide; IAA: Indole acetic acid; J2: Second-stage juvenile; Pf: Final population; PGPR: Plant growth-promoting rhizobacteria; Pi: Initial population; Rf: Reproduction factor

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Authors' contributions

The first and second authors, MR and AK, are responsible for conducting the experimental work and analysis interpretation of the data. The second author, AK, is responsible for designing and supervising the study and revising the paper scientifically. The third author, SMT, is responsible for the bacterial identification and contribution to the study. All authors read and approved the final manuscript.

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Availability of data and materials

The samples of vermicompost, bacteria, and tomato seed, used in this study, are available in the Nematode Laboratory, Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran. The datasets generated and/or analyzed during the current study are not publicly available because that the data and material are included in the dissertation of the first author and have not yet been published formally but are available from the corresponding author upon request. Accession no. of the identified bacteria are available in the NCBI database.

Ethics approval and consent to participate

The authors declare that the present article is part of the result of the research project of the first author's doctoral dissertation, which has been approved by the Vice Chancellor for Education and Postgraduate Studies of Shiraz University. In addition, they declare that all the relevant ethics issues have been considered in conducting the research and writing the manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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