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Isolation and identification of endophytic bacteria associated with kiwifruit and their biocontrol potential against *Meloidogyne incognita*

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Abstract

Background: Root-knot nematodes (RKNs) cause severe losses in kiwifruit-growing regions. The endophytic bacteria could be biological agents for nematodes management. The aim of this study was the isolation and identification of endophytic bacteria from kiwifruit orchards and the evaluation of their antagonistic ability against RKN in greenhouse conditions.

Results: In this study, the population of nematode and the bacterial strains were isolated from kiwifruit roots and leaves in the Mazandaran and Guilan provinces of Iran. Molecular experiments were conducted to identify and confirm the bacterial isolates and RKN species. Also, the effects of bacterial isolates on nematode reproduction factors (number of galls, egg masses, and second-stage juveniles, J2) and growth parameters of kiwifruit plants were determined. The RKN was confirmed as *Meloidogyne incognita* by molecular identification. Also, the endophytic bacteria were identified based on supplementary experiments and molecular analyses. A total of 31 bacterial endophytes were identified to be including 12 genera of *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Exiguobacterium*, *Sphingomonas*, *Agrobacterium*, *Variovorax*, *Pantoea*, *Microbacterium*, *Streptomyces*, *Chryseobacterium*, and *Chitinophaga*. Generally, *Bacillus* and *Pseudomonas* were the dominant genera that included 29.03 and 22.58% of total isolated bacteria, respectively. In vitro screening assays, *P. ananatis* 121.en and *P. chlororaphis* 54.en displayed considerable antagonistic ability on J2 mortality of *M. incognita* and were selected for greenhouse surveys. The isolates displayed a significant reduction in the number of galls and egg masses on roots and juvenile's population in pot soil. Moreover, 121.en and 54.en strains significantly increased growth parameters including root fresh weight and shoot fresh weight than the control kiwifruit seedlings.

Conclusions: The bacterial endophytes are safe and have a low risk of managing the RKNs and can be effective microbial bio-fertilizers for improving kiwifruit plant growth under RKNs infections.

Keywords: Root-knot nematodes, Kiwifruit, Phylogeny, *Bacillus*, Plant growth promotion

Background

Kiwifruit (family: Actinidiaceae and genus: *Actinidia* Lindl) is one of the important horticultural crops in North Iran (Mazandaran and Guilan provinces). Kiwifruit is rich in vitamin C, folic acid, antioxidant properties, high minerals and fiber, and medical applications (Pan et al. 2020).

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Root-knot nematodes (RKNs) cause extensive losses to a wide range of economical crops including *Actinidia* spp. in the world. The nematode causes gall at the root systems and impairs the absorption of water and minerals, which results in wilting, growth suppression, reduction in yield, and eventually death (Xia et al. 2019). The application of non-host plants, rotation, resistant plants, flooding, and nematicides is methods for the management of plant-parasitic nematodes. Recently, beneficial biocontrol agents such as bacteria and fungi were used for nematode's management (Tran et al. 2019). In recent years, the control of nematodes using biocontrol agents such as endophytic bacteria has increased. These endophytic bacteria are present in the inner tissues of plants, protect the plants against pathogens, affect plant growth by production of secondary metabolites, phytohormone, antibiotics, and siderophores, and trigger the plant defense response (Rat et al. 2021). Nowadays, endophytic bacteria have been used as bio-fertilizers and biocontrol agents which reduce the negative effects of chemical material on the environment and human health (Vetrivelkalai 2019).

The diversity of endophytic bacteria demonstrates that these microorganisms can relate to different plant species (Rat et al. 2021). In previous studies, endophytic bacteria were isolated and identified on the basis of 16S rRNA gene sequence from different plant species. Furthermore, the 16S rRNA gene analysis identified phyla of Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes that genera of *Pseudomonas*, *Pantoea*, *Enterobacter*, *Stenotrophomonas*, *Acinetobacter*, *Serratia* (Gammaproteobacteria), *Microbacterium*, *Staphylococcus* (Firmicutes), *Streptomyces*, *Arthrobacter* (Actinobacteria), *Bacillus*, *Paenibacillus*, and *Mycobacterium* were the abundant genera (Hardoim et al. 2015). It has been reported that the frequency of four phyla of Actinobacteria and Firmicutes at the rate of 34%, Bacteroides, alpha and beta Proteobacteria about 5% was distributed in *Pinus* species. Also, the genera of *Bacillus*, *Pseudomonas*, and *Microbacterium* are reported from pinus, watermelon, tomato, sweet corn, and pepper (Ponpandian et al. 2019). Furthermore, bacterial endophytes of *Variovorax boronicumulans* and *Agrobacterium tumefaciens* were isolated from *Phalaris arundinacea* L. (Węgrzyn and Felis 2018). The endophytes of *V. boronicumulans*, *Bacillus cereus*, and *Bacillus aryabhattai* are isolated from *Lavandula dentata* L. (Pereira et al. 2016). Moreover, *Pantoea* spp. and *Bacillus* spp. are bacterial endophytes that have been isolated from rice, maize, tomato, and medicinal plants (Abo-Elyousr and Hassan 2021). The *Pantoea* spp. was the dominant bacterial endophyte in *Pellaea calomelanos* (Sw.) Link (Mahlangu and Serepa-Dlamini 2018). According to the report of Kim et al. (2019), Proteobacteria classes (α, β, and γ) were the most dominant groups in kiwifruit endophytic communities. Also, Cho et al. (2018) reported that the endophytic *Pseudomonas* is the dominant genus in kiwifruit.

Moreover, there are many reports about the application of bacterial endophytes as biological control agents (Vetrivelkalai 2019). For instance, it has been reported that endophytic *Bacillus amyloliquefaciens* decreased decay rate (73.12%) against *Botryosphaeria dothidea* in kiwifruit (Pang et al. 2021). *Bacillus halotolerans* LYSX1 has an antagonistic ability for the biocontrol of *Meloidogyne javanica* (Treub 1885) Chitwood 1949 in *Lycopersicon esculentum* cv. Sufen (Xia et al. 2019). *Streptomyces* sp. CBG9 has significant nematicidal ability against *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 in *Coffea canephora* Pierre ex A. Froehn (Hoang et al. 2020). Although there are studies on endophytic microorganisms in a wide variety of plants, only limited information is available from endophytic bacteria in kiwifruit. Therefore, the aim of this study was the isolation and identification of endophytic bacteria communities from kiwifruit leaves and roots and the evaluation of their antagonistic potential against *M. incognita*.

Methods

Preparation and identification of RKNs

The population of RKNs was collected from kiwifruit's roots in the north of Iran, Mazandaran Province. The single egg masses were propagated on roots of susceptible tomato (Early Urbana variety) in a greenhouse (temperature of 25 °C ± 2 and RH of 70%). After 50 days, the pure nematode population was extracted from tomato roots. Molecular identification of nematode species was performed using species-specific primers of SEC-1F (5'GGGCAAGTAAGGATGCTCTG3')/SEC-1R (5'GCA CCTCTTTCATAGCCACG3') (Tesařová et al. 2003) for *M. incognita*. The DNA extraction was carried out using a modified method of Silva et al. (2000). The polymerase chain reaction (PCR) was done in 50 µl solutions containing 25 µl of 2 × master mix, 1 µl of each primer (10 µm), and 50 ng DNA template. The reaction conditions include 3 min at 94 °C, 35 cycles consisting of 30 s, 94 °C; 30 s, 56 °C; and 1 min, 72 °C, with a final extension of 7 min at 72 °C. The PCR products were separated on an agarose gel (1%) and visualized under UV light.

Plant sampling, isolation, and identification of endophytic bacteria

A total of 200 plant samples (leaf and root) were collected from kiwifruit vineyards in the north of Iran both Mazandaran and Guilan provinces. The samples were transferred immediately to the laboratory of citrus and subtropical fruits research center, Agricultural Research

Education and Extension Organization (AREEO), Ramsar, Iran.

Isolation of endophytes was done according to Wicaksono et al. (2018) method. The samples were sterilized with 96% ethanol for 10 s, 2% sodium hypochlorite solution for 3 min followed by washing 3 times (one min each time) with sterile distilled water in a laminar flow cabinet. The disinfected tissues were fragmented in sterilized water for 30–40 min and 30 µL of suspension was cultured on sucrose nutrient agar (NAS) medium after serial dilution. Also, 100 µl of the last wash was transferred to Luria–Bertani (LB) as a control (Taechowisan et al. 2003). The plates were incubated at 25 °C for 7 days. The colonies were re-cultured on NAS plates until pure colonies were obtained. Single colonies were stored in 60% glycerol and were stored at − 80 °C for future studies.

A total of 100 isolates were grouped based on morphological features such as filamentous shapes, rough and smooth texture, flat and irregular-edged, and white, yellow, yellow to orange, cream, lemon yellow, and orange colors and gram reaction. For molecular identification, 31 colonies with morphological differences were selected. Bacterial genomic DNA was extracted using the cells lysis protocol (Keegan et al. 2005). The extracted DNA was used for the amplification of the 16S rRNA gene with the universal bacterial primers FD1 (5'AGAGTTTGATCC TGGCTCAG3') and RP2 (5'ACGGTTACCTTGTTA CGACTT3') (Weisburg et al. 1991). PCR was performed in 50 µl reactions containing 50 ng DNA template, 1 µl of each primer (10 pmol × μl^{-1}), 25 µl Taq DNA Polymerase 2 × Master Mix RED (Ampliqon, Cat. No. A180301) using a MJ RESEARCH PTC-200 thermal cycler. The amplification program contains initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 50 s, annealing at 55 °C for 45 s, and extension at 72 °C for 90 s, and a final extension for 3 min at 72 °C. The PCR products (~ 1500 bp) were separated using agarose gel 1% with TBE buffer 1X, stained with SinaClon DNA safe Stain (Cat. No. EP5082, Co., Tehran, Iran), and visualized under UV light. The amplicons were sequenced and the results were compared to the sequences deposited in the national center for biotechnology information database (www.ncbi.nlm.nih.gov). The obtained sequences were aligned using the multiple sequence alignment in MEGA X software. Phylogenetic analysis was carried out using the maximum likelihood method with bootstrapping (1000 replications). Then sequences were deposited in GenBank for obtaining accession numbers.

In vitro nematicidal activity

For evaluation of antagonistic effects of endophytic isolates on mortality of J2s, the suspension of freshly hatched juveniles was used. The isolated eggs from the tomato roots were incubated at 27 ± 2 °C, and freshly hatched juveniles were used for the experiment.

Anti-nematode activity: One ml suspension of selected bacterial isolates (10^8 CFU/ml) was added to 1 ml of 50 J2s and kept at room temperature. Nematode (J2) mortality was recorded at 48 and 72 h after treatment under a stereoscopic microscope with a magnification of 4×. The negative control with sterile water was performed under the same conditions. Each treatment included five replications and the experiment was repeated thrice. The number of dead juveniles was counted 1–3 days and the mortality ratio of J2s was calculated.

Greenhouse experiments

The endophytic bacterial isolates (121.en and 54.en) with the highest nematicidal activity in the in vitro assays were selected for the greenhouse experiments. For this purpose, 40 ml of the bacterial suspension (1×10^8 CFU/ml) were treated to six-month-old seedlings of *Actinidia chinensis* var. *deliciosa* (A. Chev) cv. Hayward and after 2 days, seedlings were inoculated with 2000 J2s of *M. incognita* and kept in a greenhouse at 25 ± 4 °C. The treatments were including the seedlings treated only with sterile water (Control); inoculated only with nematode; inoculated with nematode and treated with 121.en; and inoculated with nematode and treated with 54.en. The experiment was carried out in a randomized block design with five replications and repeated thrice. After 50 days, the number of galls and egg masses, fresh and dry weights of shoot and root, and juvenile population of nematodes in the soil were measured.

Statistical analysis

Data were analyzed using SAS version 9.1 software with a one-way variance analysis (ANOVA) test. The mean ± standard deviation ($X \pm SD$) was expressed in all experimental data. The significance of differences ($P < 0.05$) within treatments was determined using Duncan's test.

Results

Identification of *M. incognita*

PCR was performed using specific primers of Inc-k14 and fragments of about 502 bp were produced for *M. incognita* (Fig. 1).

Identification of endophytic bacteria from kiwifruit

The phylogenetic analysis grouped the isolates into six groups and 12 genera (*Bacillus*, *Pseudomonas*, *Chryseobacterium*, *Microbacterium*, *Pantoea*, *Streptomyces*, *Sphingomonas*, *Agrobacterium*, *Chitinophaga*, *Staphylococcus*, *Variovorax, and Exiguobacterium*) (Table 1 and Fig. 2). The accession numbers and relationships

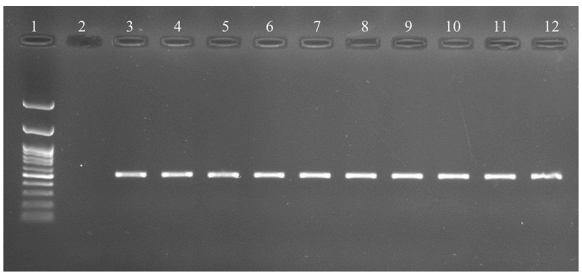

Fig. 1 Amplification of PCR products using specific primers of Inc-k14 on agarose gel 1%. Lines; 1: 100 bp DNA marker, 2: Negative control, 3–12: Collected samples of *Meloidogyne* (pure nematode population)

between genera or species of these endophytic bacteria with sequences deposited in the GenBank database are demonstrated in Table 1. All of the consensus sequences obtained in this study presented a high similarity (up to 97%) with the closest related sequences deposited in the GenBank. Phylogenetic analysis of these strains is demonstrated in Fig. 2.

Results allowed us to classify the isolated kiwifruit endophytic bacteria into 6 distinct groups. Group I was belonging to the Firmicutes phylum. This group includes isolates that belonged to the *Bacillus* (123.en, 60.en, 1.en, 10.en, 31.en, Q.en, M.en, N.en, and K.en), *Staphylococcus* (27.en and X.en) *Exiguobacterium* (O.en), which was the most dominant groups in this study. Group II (α-Proteobacteria phylum) including *Agrobacterium* (isolates 2.en and 19.en) and *Sphingomonas* (isolate 5.en), Group III (β-Proteobacteria phylum), two bacteria belonged to the *Variovorax* (isolates 48.en and 53.en), Group IV (Gammaproteobacteria phylum), seven bacteria belonged to the *Pseudomonas* (isolates 54.en,

Table 1 Identification of isolated endophytic bacteria from kiwifruit

Groups	Characteristics of isolates	Strain	Accession number	Source
Group I Firmicutes	*Bacillus altitudinis*	123.en	MW282048	Root
	Bacillus safensis	Q.en	OK415424	Root
	Bacillus (*Priestia*) *megaterium*	31.en	OK560186	Leaf
	Bacillus (*Priestia*) *megaterium*	60.en	OK560185	Leaf
	Bacillus halotolerans	1.en	OK415792	Leaf
	Bacillus altitudinis	10.en	OK415851	Leaf
	Bacillus sp.	M.en	OK416036	Root
	Bacillus sp.	N.en	OK416060	Root
	Bacillus sp.	K.en	OK416021	Root
	Staphylococcus (*Mammaliicoccus*) *sciuri*	27.en	OK560183	Leaf
	Staphylococcus (*Mammaliicoccus*) *sciuri*	X.en	OK560184	Root
	Exiguobacterium acetylicum	O.en	OK394042	Root
Group II α-Proteobacteria	*Sphingomonas* sp.	5.en	OK394039	Leaf
	Agrobacterium tumefaciens	2.en	OK398363	Leaf
	Agrobacterium tumefaciens	19.en	OK398382	Leaf
Group III β-proteobacteria	*Variovorax boronicumulans*	48.en	OK396002	Leaf
	Variovorax boronicumulans	53.en	OK396017	Leaf
Group IV Gammaproteobacteria	*Pseudomonas fulva*	28.en	OK415025	Leaf
	Pseudomonas chlororaphis	14.en	OK415300	Root
	Pseudomonas chlororaphis	41.en	OK415301	Leaf
	Pseudomonas chlororaphis	51.en	OK415297	Root
	Pseudomonas chlororaphis	54.en	OK415343	Leaf
	Pseudomonas chlororaphis	P.en	OK415418	Root
	Pseudomonas psychrotolerans	24.en	OK415027	Root
	Pantoea ananatis	121.en	OK394040	Leaf
Group V Actinobacteria	*Microbacterium foliorum*	3.en	OK398243	Leaf
	Microbacterium foliorum	6.en	OK398247	Leaf
	Streptomyces sp.	J.en	OK398274	Root
Group VI Bacteroidetes	*Chryseobacterium indologenes*	44.en	OK398275	Leaf
	Chryseobacterium sp.	7.en	OK398276	Leaf
	Chitinophaga sp.	43.en	OK398362	Leaf

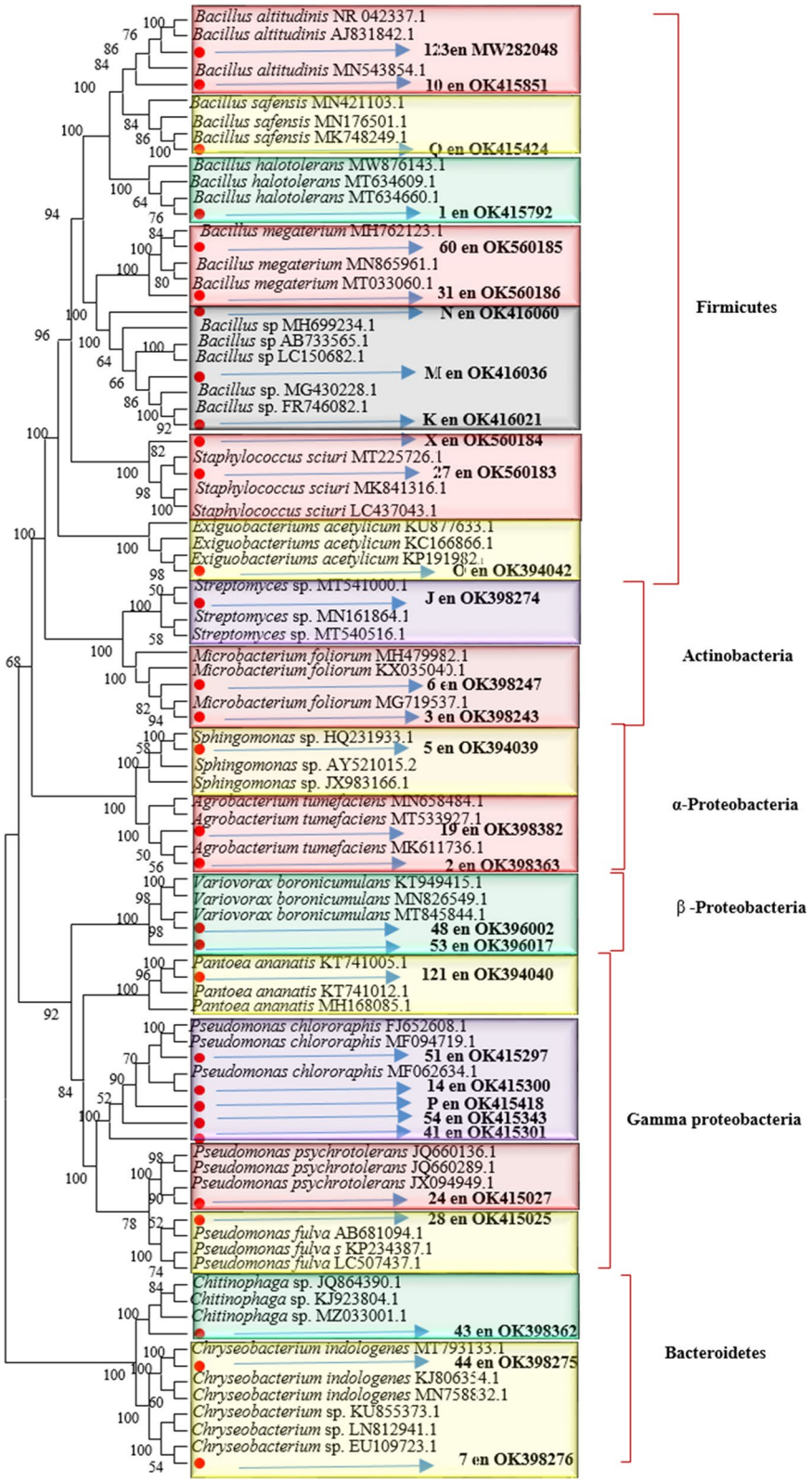

Fig. 2 Phylogenetic tree of isolated endophytic bacteria from kiwifruit based on the 16 s rRNA gene sequences constructed in the MEGA X program based on the maximum likelihood method and bootstrap values (values > 50%) based on 1000 replications. *Bacillus megaterium* and *Staphylococcus sciuri* have changed to *Priestia megaterium* and *Mammaliicoccus sciuri*

41.en, 14.en, 24.en 28.en, 51.en, and P.en) and one bacterium belonged to the *Pantoea* (isolates 121.en), Group V (Actinobacteria phylum), two bacteria belonged to the *Microbacterium* (isolates 3.en and 6.en), and one bacteria belonged to the *Streptomyces* (isolates J.en), and Group VI (Bacteroidetes phylum) contained two bacteria belonged to the *Chryseobacterium* (isolates 7.en and 44.en) and one bacteria belonged to the *Chitinophaga* (43.en) (Fig. 2).

Among these groups, 12 isolates (29.03%) were gram-positive, rod shapes, spore-forming, and irregular-edged belonging to the *Bacillus* genus. Furthermore, seven isolates (22.58%) were gram-negative, motile, non-spore-forming, catalase-positive, and oxidase negative identified as *Pseudomonas* spp. Thus, according to the observed results, *Bacillus* and *Pseudomonas* were the most dominant genera among isolates and the other genera had a frequency of between 3 and 10% (Fig. 3).

In vitro activity

Statistical analyses indicated significant differences among bacterial isolates in terms of J2s mortality. The results of some isolates are given in Table 2. The mean comparison of isolates data indicated that *P. ananatis* 121.en and *P. chlororaphis* 54.en had the most effect on J2s mortality and increased the percentage of J2 mortality to 83.3 and 79.26% after 72 h., respectively (Table 2). Thus, these two isolates were selected for greenhouse experiments.

Greenhouse experiment

Two isolates of *P. ananatis* 121.en and *P. chlororaphis* 54.en were selected for greenhouse experiments based on the high antagonistic ability on J2s mortality in vitro. Results of the greenhouse experiments demonstrated that endophytic bacteria had positive effects in kiwifruit seedlings inoculated with *M. incognita*. Indeed, there were significant differences among treatments in nematode reproductive parameters and plant growth parameters in comparison with control plants. The 121.en and 54.en isolates were able to reduce the number of galls and egg masses in the roots of kiwifruit seedlings infected with the nematode. The reduction in the number of galls was 75.11% (121.en) and 74.83% (54.en), and the reduction in the number of egg masses was 86.63% (121.en) and 86.80% (54.en). Also, those were able to reduce the J2s population in the soil, formed by 92% (121.en), and 92.59% (54.en) (Fig. 4). Furthermore, seedlings treated with these three isolates promoted kiwifruit plant growth including the root and shoot fresh weight and subsequently root and shoot dry weight as compared to positive control seedlings (Table 3).

Table 2 Mean comparison of percentage of mortality of J2s in some bacterial isolates

Treatments	J2 mortality rate (%) after 48 h	J2 mortality rate (%) after 72 h
Pantoea ananatis 121.en	70.00^{a}	83.33^{a}
Pseudomonas chlororaphis 54.en	65.86^{b}	79.26^{b}
Bacillus (*Priestia*) *megaterium* 31.en	62.13^{b}	73.80^{b}
Agrobacterium tumefaciens 19.en	62.06^{b}	71.40^{b}
Chryseobacterium indologenes 44.en	60.93^{b}	70.80^{cb}
Pseudomonas fulva 28.en	62.93^{b}	70.33^{cb}
Bacillus safensis Q.en	62.00^{b}	69.80^{cb}
Pseudomonas chlororaphis P.en	61.86^{b}	65.73^{cb}
Control	4.60^{c}	7.53^{c}
F value	777.15	893.39
Pr > F	< 0.0001	< 0.0001

Means comparisons evaluated by the same letter are not significantly different based on Duncan's test ($P < 0.05$). Data are averages of five replicates and three repetitions

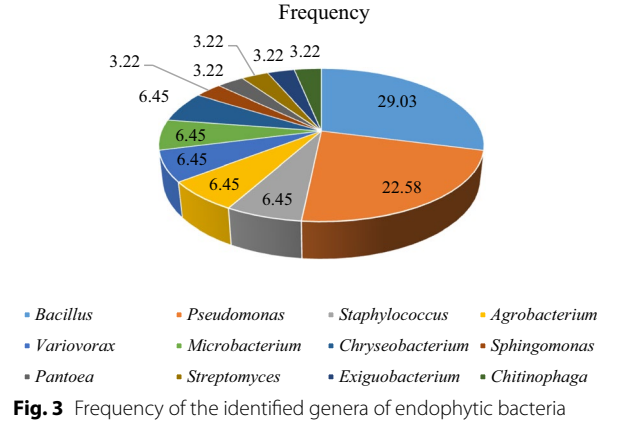

Fig. 3 Frequency of the identified genera of endophytic bacteria from kiwifruit

Discussion

This study focused on the diversity of endophytic bacteria and the evaluation of biological control efficacy against *M. incognita*. A few plants have been studied for their endophytic organisms (Sharma et al. 2018). There are many reports on the isolation of endophytic bacteria from kiwifruit. It has been founded that *Pseudomonas* (Xie et al. 2021) and *Stenotrophomonas* (Cho et al. 2018) were the dominant genera in kiwifruit. Some studies have shown the antagonistic ability of endophytic bacteria against plant pathogens in kiwifruit. The endophytes including *Pseudomonas fluorescens*, *P. putida*, *P. mendocina*, *Pantoea agglomerans*, and *Kluyvera intermedia* had inhibitory ability against *P. syringae* pv. *actinidiae* in kiwifruit (Tontou et al. 2016b). Also, the *P. synxantha*

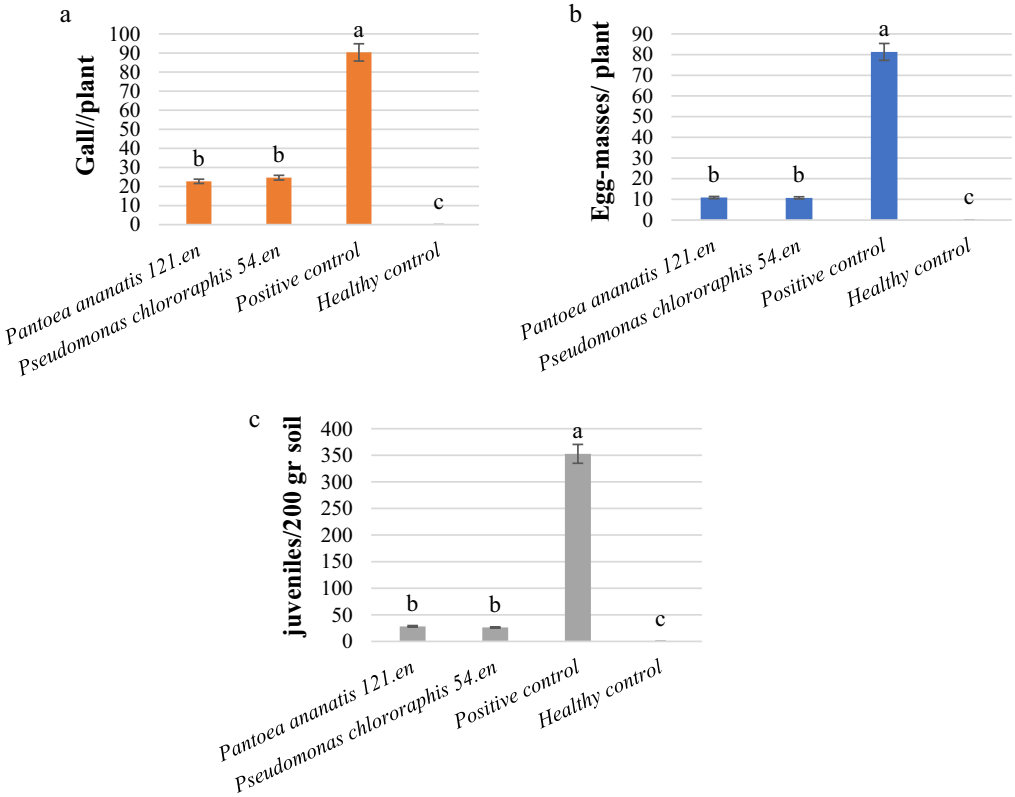

Fig. 4 Mean comparisons of nematode reproductive factors in treatments; **a** and **b**: The number of gall and egg masses in root system; **c**: The number of juveniles in 200 gr of soil. Positive control (nematode only) and negative control (without bacteria and nematode)

Table 3 Mean comparison of plant growth parameters in greenhouse experiment

Treatment	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
121.en[1] + Nematode	9.25[b]	0.512[b]	34.70[a]	6.084[a]
54.en[2] + Nematode	9.19[b]	0.514[b]	34.67[a]	6.081[a]
Positive control	5.99[c]	0.316[c]	25.617[c]	4.946[c]
Negative control	11.67[a]	1.206[a]	30.142[b]	5.524[b]
F value	35.17	37.24	23.11	9.53
Pr > F	< 0.0001	< 0.0001	0.0287	0.3706

Data are mean of five replications and three repetitions. Letters following numbers indicate significant differences ($P < 0.05$). Negative control: sterile distilled water; positive control: inoculated with just *Meloidogyne incognita*.

[1] : Pantoea ananatis, [2]: Pseudomonas chlororaphis

was effective against *P. syringae* pv. *actinidiae* in *Actinidia chinensis* (Tontou et al. 2016a). Previous findings also confirmed the great effects of endophytic bacteria on controlling nematodes. For example, it was reported that *B. megaterium* DS9 reduced the nematode population and affected pepper growth promotion (Tran et al. 2019). In the other research, endophytic *Streptomyces* sp. isolated from banana had high antagonistic potential against *M. javanica* (Su et al. 2017). Vetrivelkalai (2019) reported that isolates of *Bacillus* sp. and *Pseudomonas* sp. reduced the number of egg masses and *M. incognita* population in the soil. In another research, Sharma and Sharma (2017) reported *Pseudomonas* sp. reduced the nematode infection and increased the growth parameters

in tomato plants. Also, Khanna et al. (2019) revealed that *P. aeruginosa* increased root dry weight and fresh weight 17.5 and 16.4% in *Lycopersicon esculentum* Mill. seedlings. Indeed, the results of this study were in agreement with these reports that 121.en and 54.en isolates significantly reduced the number of galls and egg masses and promoted plant growth in greenhouse assays.

The 16S rRNA gene sequencing is important for phylogeny studies and is a useful tool for the identification of bacterial endophytes to subspecies level in a variety of plants ((Liaqat and Eltem 2016). The results of the present study showed that the most dominant genus was *Bacillus*, with a frequency of 29.03%, which was not organ-specific and isolated from leaf and root tissues, and the strains of *B. altitudinis* 123.en and 10.en, *B. safensis* Q.en, *B. megaterium* 31.en and 60.en, *B. halotolerans* 1.en and *Bacillus* sp. K.en, M.en, and N.en, reported from the *Bacillus* genus. The *Bacillus* genus is an endophytic bacterium from the phylum Firmicutes and the family of *Bacillaceae*. The *Bacillus* species are important due to auxin, gibberellin, and siderophore production, phosphate solubilization, and the ability to adapt to drought (Dias et al. 2009). This genus is effective for the biological control of plant diseases against *Meloidogyne hapla* Chitwood 1949, *Fusarium oxysporum*, and other pathogens (Ma et al. 2013). There are reports on the biological control of plant diseases using endophytic bacteria. For example, the *B. altitudinis* with the production of main metabolites such as cyclic lipopeptides is a proper candidate as a bio-fertilizer and plant growth promoter in sustainable agriculture (Zhang et al. 2021). The *B. halotolerans* Y6 with overexpressing β-glucanase inhibits the mycelial growth of *Verticillium dahlia* because the production of secondary metabolites is important in biological control (Zhang et al. 2019). *B. safensis* ZY16 isolated from *Chloris virgata* Sw. with the production of biosurfactants and degradation of hydrocarbons causes plant growth promotion (Wu et al. 2019). The endophytic *B. megaterium* RmBm31 isolated from *Retama monosperma* (L.) Boiss. via solubilization of phosphate and production of indole-3-acetic acid (IAA) causes plant growth-promoting (Dahmani et al. 2020). The second genus in this study was the *Pseudomonas*, with a frequency of 29.03% that strains of *P. fulva* 28.en, *P. chlororaphis* 14.en, 41.en, 51.en, 54.en, P.en and *P. psychrotolerans* 24.en, reported from *Pseudomonas* genus. The *Pseudomonas* from the class Gammaproteobacteria and the family *Pseudomonadaceae* are reported in most plants. *Pseudomonas* genus due to the production of various antibiotics including HCN, 2, 4-diacetylphloroglucinol, and pyrrolnitrin is affected plant growth (Whipps 2001). It has been reported that endophytic *P. fulva* strain MRC41 isolated from maize with activities of plant growth promotion and drought tolerance helped plant disease management (Sandhya et al. 2017). *P. chlororaphis* strains due to the production of antibiotics and trigger of systemic resistance protect plants against plant diseases. The endophytic *P. psychrotolerans* isolated from *Taxus chinensis* (Rehder & E.H.Wilson) Rehder via the production of antioxidants has antagonistic activity and is effective for plant growth promotion (Fidan and Zhan 2019). The third genus in this study with a frequency of 6.45% was from the *Chryseobacterium* genus, family *Flavobacteriaceae*. An endophytic *Chryseobacterium* sp. GSE06 isolated from *Cucumis sativus* L. was a biocontrol agent against *Phytophthora capsici*. This genus has the ability of colonization, plant growth promotion, and antimicrobial activity (Jeong et al. 2016). Eke et al. (2019) reported that the endophytic *Ch. indologenes* isolated from *Euphorbia trigona* Mill was effective for tomato growth promotion. The fourth genus, with a frequency of 6.45%, belongs to the genus *Microbacterium* from the family of *Microbacteriaceae* that in this study strains of *M. foliorum* 3.en and 6.en reported from *Microbacterium* genus. The *Microbacterium* strains with produce secondary metabolites (antibiotics, solubilize Potassium, pigments, and siderophores) have an important role in biological control (Corretto et al. 2020). For example, the *M. foliorum* CT10 isolated from tomato stems and leaves was effective against *F. oxysporum* and *Botrytis cinerea* (Hernández-Pacheco et al. 2021). The fifth genus was the *Staphylococcus*, with a frequency of 6.45% that in this study, strains of *S. sciuri* 27.en and X.en were reported from *Staphylococcus* genus. They belong to the family *Staphylococcaceae* that have salt tolerance potential and influence in the release of growth regulators and produce gibberellin, IAA, protease, chitinase, and siderophore (Alijani et al. 2019). The endophytic *Staphylococcus sciuri* reported from roots *Leptochloa fusca* L. by phosphate solubilization and production of phytohormone regulates plant development. However, this genus plays the important role in the biological control of plant pathogens (Dutta et al. 2017), for example, *S. sciuri* MarR44 as an endophytic bacterial isolated from strawberry due to the production of volatile compounds had antifungal activity against strawberry anthracnose (Alijani et al. 2019). The sixth genus was the *Variovorax* (family of *Comamonadaceae*), with a frequency of 6.45% that in this study, strains of *V. boronicumulans* 48.en and 53.en were reported from *Variovorax* genus. This genus has an important role in microbe–plant interactions. *V. boronicumulans* regulates hormone IAA levels in plants (Sun et al. 2018). It has been reported that the *V. boronicumulans* CGMCC4969 promotes plant growth by producing siderophores, hydrogen cyanide, ammonia, and secreting salicylate (Liu et al. 2013). The seventh genus, with a

frequency of 3.22%, belongs to the genus *Chitinophaga* from the family of *Chitinophagaceae* that in this research, the strain of *Chitinophaga* sp. 43.en, reported from *Chitinophaga* genus. This genus produces secondary metabolites with antimicrobial activity and is an effective endophyte for the biological control of pathogens such as *Rhizoctonia solani* (Carrión et al. 2019). It has been reported that in the *Chitinophaga* MR33 strain via multifunctional activities such as phosphate solubilization, protease, and chitinolytic activity, IAA production is effective for plant growth promotion (Chimwamurombe et al. 2016). The eighth genus was the *Agrobacterium*, with a frequency of 6.45% that in this study, strains of *A. tumefaciens* 2.en and 19.en were reported from *Agrobacterium* genus. The genus belongs to the family of *Rhizobiaceae*. There are reports of endophytic *A. tumefaciens* strains with the potential of plant-growth-promoting that have been isolated from various plants. The endophytic *A. tumefaciens* CCNWGS0286 isolated from the *Robinia pseudoacacia* L. had a key role as plant-growth-promoting (Hao et al. 2012). Too, *A. tumefaciens* CR22 isolated from tomato roots effective against *F. oxysporum* (Hernández-Pacheco et al. 2021). The ninth genus, with a frequency of 3.22%, belonged to the genus *Pantoea* from the family of *Erwiniaceae*. In this study, the strain of *P. ananatis* 121.en was reported from *Pantoea* genus. It has been reported that *P. ananatis* D1 promotes plant growth by producing siderophore, 1-aminocyclopropane-1-carboxylic acid deaminase, and indole-3-acetic acid (Lu et al. 2021). The tenth genus, with a frequency of 3.22%, belongs to the genus *Sphingomonas* from the family of *Sphingomonadaceae*. The strain of *Sphingomonas* sp. 5.en was reported from *Sphingomonas* genus. The *Sphingomonas* strains have beneficial properties as endophytes in various plants such as papaya (Rivarez et al. 2021), and *Allium tuberosum* (Huang 2019). *Sphingomonas* sp. as a common plant bacterial endophyte is beneficial for plants by increasing of producing phytohormone. The eleventh genus, with a frequency of 3.22%, belongs to the genus *Streptomyces* from the family of *Streptomycetaceae*. The strain of *Streptomyces* sp. J.en reported from *Streptomyces* genus. *Streptomyces* bacteria as biocontrol agents produced secondary metabolites such as antimicrobials volatile compounds, IAA hormone, and plant protection from biotic stresses, which colonize plant roots. They stimulate plant growth and control different bacterial and fungal pathogens (Vurukonda et al. 2021). The last genus, with a frequency of 3.22%, belongs to the genus *Exiguobacterium* from the family of *Bacillaceae*. The strain of *E. acetylicum* O.en was reported from *Exiguobacterium* genus. The *Exiguobacterium acetylicum* had the potential of phosphate solubilizing, ability of enzyme production, plant growth promotion and increases plant tolerance to cold (Selvakumar et al. 2010). The *E. acetylicum* was isolated from C4 plants (Girsowicz et al. 2019). It has been reported that *E. acetylicum* suppressed the growth of the plant pathogens *F. oxysporum*, *Sclerotium rolfsii*, *R. solani*, and *Pythium* sp (Selvakumar et al. 2010).

Generally, to manage nematodes, two stages of second-stage juvenile and eggs should be controlled because these are important in their life cycle; interruption in these stages results in a decrease in nematode populations (Xiang et al. 2018). Endophytic bacteria by the production of enzymes, HCN, hormones, secondary metabolites, and induction of plant resistance suppress pathogens. These bacteria by producing chitinase, protease, catalase, lipase enzymes, and HCN can kill nematodes. Also, they promote plant growth by dissolving phosphorus and stabilizing nitrogen (Ma et al. 2016). In the present study, isolation of endophytic bacteria was performed from healthy kiwifruit trees in nematodes-infected orchards. In these orchards, some trees were unaffected by nematodes and remained healthy. The findings may show that this can be due to beneficent microorganisms such as endophytes in the plant and rhizosphere. The greenhouse experiments were conducted to assess the biological control efficacy of endophytic bacteria against RKN in kiwifruit seedlings. The results showed that the treatment of endophytic bacteria *P. ananatis* 121.en and *P. chlororaphis* 54.en reduced the numbers of galls and egg masses and promoted the growth of kiwifruit plants in compared with control plants. Also, the rate of J2 mortality increased in the soil 50 days after inoculation. Similar results were indicated by Vetrivelkalai (2019) who reported that the number of galls, egg masses, and population of RKNs were significantly reduced and the plant growth parameters were promoted in plants treated with endophytic bacteria of *Pseudomonas*, *Pantoea*, and *Bacillus*. Therefore, obtained results indicated that endophytic bacteria of *P. ananatis* 121.en and *P. chlororaphis* 54.en can be potential agents against *M. incognita* and provide a useful option for the selection of kiwifruit growth-promoting microorganisms.

Conclusions

The results showed that endophytic bacteria isolated from kiwifruit vine had a high diversity genetically and belonged to different phylogenetic groups from genera *Bacillus*, *Pseudomonas*, *Chryseobacterium*, *Microbacterium*, *Pantoea*, *Streptomyces*, *Variovorax*, and *Exiguobacterium*. This diversity indicates the fact that kiwifruit vine habitats for a variety of gram-positive and gram-negative endophytic bacteria. Moreover, the results showed that the antagonistic ability of two isolates *P. ananatis* 121.en and *P. chlororaphis* 54.en against *M. incognita* resulted in a reduction in the number of gall and egg masses in

the roots, a decrease in the J2 population in the soil, and improvement in plant growth parameters. Thus, these endophytic bacteria can be both plant growth stimulators and biocontrol agents against root-knot nematodes. On the other hand, these isolates can be a good candidate for microbial formulation to manage pathogens and improve plant growth. Therefore, large-scale field experiments should be performed in the future.

Abbreviations

RKNs: Root-knot nematodes; J2: Second-stage juvenile; AREEO: Agricultural Research Education and Extension Organization; NAS: Sucrose nutrient agar; LB: Luria–Bertani; PCR: Polymerase chain reaction; IAA: Indole-3-acetic acid.

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Author contributions

SJ (Plant Pathology-Nematology), MG (Plant Pathology-Bacteriology), and MG (Horticultural Science) were the advisor and the supervisors of the thesis; SNB carried out all the experiments (Plant Pathology Student—PhD thesis). All authors read and approved the final manuscript.

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Availability of data and materials

All data generated during the current study are included in this article, and sequence data generated are available as nucleotide sequence in the NCBI GenBank.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript has not been published elsewhere.

Competing interests

The authors declare that they have no competing interests.

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