Cetintas et al. Egyptian Journal of Biological Pest Control (2018) 28:7
DOI 10.1186/s41938-017-0008-x

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Effect of some plant growth-promoting rhizobacteria strains on root-knot nematode, *Meloidogyne incognita*, on tomatoes

Ramazan Cetintas*, Mustafa Kusek and Shirkoo Ameen Fateh

Abstract

Root-knot nematode, *Meloidogyne incognita*, is one of the main problems in vegetable-growing regions, decreasing yield quality and quantity worldwide. Root-knot nematode management tactics mostly involve chemical control which is a threat to the environment, consequences to human health. Biological control of nematodes is considered to be one of the best alternatives to the chemical control. In this study, the effect of some bacterial isolates against *M. incognita* was determined on tomato in the greenhouse. The trial was designed as a randomized complete block, consisting of 15 plant growth-promoting rhizobacteria (PGPR) strains and 2 control groups (−, +) with 10 replications, total of 170 pots. Two days after transplanting the bacteria-treated tomato (*Solanum lycopersicum* cv. 56-56 F1) seedlings in the sterile soil in pots, the plants were inoculated with 1000 eggs or J2s of *M. incognita*/pot. At the end of a 90-day plant-growing period, isolate ZHA90 of *Bacillus pumilus* decreased plant root galling which, in turn, increased plant height, shoot fresh and dry weight, and root fresh weight. Isolates ZHA296 and ZHA178 of *Paenibacillus castaneae* reduced the number of egg masses and root galling with no effects on plant growth compared to the control (+). While the isolate ZHA17 of *Mycobacterium immunogenum* increased plant height and shoot fresh weight, ZHA57 of the same bacterium enhanced significantly only plant height. Results indicated that among 15 bacterial strains studied, ZHA296 and ZHA178 of *P. castaneae* and ZHA17 and ZHA57 of *M. immunogenum* were identified as the promising biocontrol agents for the future nematode management tactics.

Keywords: Biological control, *Meloidogyne incognita*, Rhizobacteria, Tomato

Background

Root-knot nematodes, *Meloidogyne* spp., are one of the main pest groups causing serious crop losses in agricultural areas. Over 90 species of genus *Meloidogyne* were recorded (Jones et al., 2013), and the most common root knot nematode species are the following: *Meloidogyne javanica*, *Meloidogyne incognita*, *Meloidogyne hapla*, and *Meloidogyne arenaria*. The studies concluded that these nematodes can infect more than 3000 host plant species in agriculture (Jung and Wyss, 1999; Hussey and Janssen, 2002; Abad et al., 2003. Consequentially, among the many nematodes, the groups having some economic impact, *Meloidogyne* spp., are responsible for a large part of the annual 100 billion dollar losses attributed to nematode damage worldwide (Ralmi et al., 2016). The yield losses in vegetables such as tomato, melon, and eggplants exceed 30% (Sikora and Fernández, 2005).

The control strategies of plant parasitic nematodes in agriculture fields mostly include chemical, biological, physical, and cultural measures along with the use of resistant cultivars. The use of nematicides is not preferred because of environmental contamination and toxicity. Biological control of nematodes considered to be the best alternative to chemical control solely or in the Integrated Pest Management (IPM) concept. Biological control of plant parasitic nematodes involves mostly antagonistic fungi and bacteria. Most of endophytic

* Correspondence: cetintas@ksu.edu.tr
Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi, Kahramanmaras, Turkey

bacteria can persist in the rhizosphere of many plant species, including vegetables and fruits without any adverse effect on overall plant health (Hallmann, 2001). Rhizobacteria could be used as biological control agents since they are colonizers of the root zone as soil microflora and sustain a positive effect on plant growth (Kloepper et al., 1992). Life cycle and development of most plant parasitic nematodes occur in the rhizosphere of host plants, where they closely interact with existing antagonists (Insunza et al., 2002). Rhizobacteria is categorized as plant health-promoting rhizobacteria (PHPR) (Sikora, 1988) or plant growth-promoting rhizobacteria (PGPR) (Kloepper et al., 1992). PGPR are free-living bacteria group that can colonize in the rhizosphere zone and stimulate root growth. The role of PGPR in the biological control concept was taken into consideration in some of recent studies. Albeit some Rhizobacteria species such as *Bacillus sphaericus*, *Bacillus subtilis*, and *Pseudomonas fluorescens* are reported to antagonize some of plant-parasitic nematodes (Tian et al., 2007), little information is available on the efficacy of PGPR bacteria on root-knot nematodes in vegetables.

This study was conducted to determine the effect of 15 PGPR strains against *Meloidogyne incognita* on tomato in the greenhouse.

Methods

Experimental design

The trials were conducted in a greenhouse located in Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey. The size of plastic pots used in the trials was about (2 L in capacity and 15 cm in diameter). The soil used consisted of 65% sand, 20% clay, 12% silt, and less than 3% organic matter. The trial was designed as a randomized complete block, consisting of 15 plant growth-promoting rhizobacteria (PGPR) strains (Table 1) and 2 controls: (−) without nematodes and bacteria together and (+) with nematodes but without bacteria. The experiment consisted of 10 replications with a total of 170 pots. Plant host used in the study was *M. incognita*-susceptible tomato, *Solanum lycopersicum* cv. 56-56 F1.

Bacteria source and preparation

The bacterial strains were obtained from the bacterial collection in the Bacteriology Laboratory, Department of Plant Protection, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey. Vegetative cells of each bacterium were obtained by culturing them on nutrient agar medium (Merck, 1.05450) in disposable Petri dishes. They were kept at 25 ± 2 °C for 48 h; thereafter, each bacterial stain was suspended by saline buffer and arranged to 1×10^{10} cfu by spectrophotometer at 600-nm wavelength. Four-week-old tomato seedlings were treated by soaking in each of 15 bacterial cell suspensions individually for 10 min and transplanted promptly into the pots.

Table 1 The plant growth-promoting rhizobacteria (PGPR) strains used as treatments in the experiment

Strain name	Bacteria species
ZHA246	*Mycobacterium confluentis*
ZHA235	Unidentified
ZHA90	*Bacillus pumilus*
ZHA17	*Mycobacterium immunogenum*
ZHA212	*Paenibacillus castaneae*
ZHA215	*Paenibacillus castaneae*
ZHA287	*Bacillus subtilis ss subtilis*
ZHA579	Unidentified
ZHA191	*Pseudomonas fluorescens*
ZHA308	*Pseudomonas viridilivida*
ZHA178	*Paenibacillus castaneae*
ZHA57	*Mycobacterium immunogenum*
ZHA569	*Tsukamurella paurometabola*
ZHA296	*Paenibacillus castaneae*
ZHA88	*Paenibacillus castaneae*

Nematode inoculum source

Meloidogyne incognita population used in this study was originally collected from field populations in Kahramanmaras. The nematode species was identified using perineal patterns and esterase phenotypes. A single egg mass was obtained from identified population reared on nematode-susceptible tomatoes in a greenhouse. In order to provide enough number of nematodes for the study, tomato plants were inoculated by the nematodes and reared in a greenhouse for 90 days. Then, the infected plants were cut at soil level, and the roots were washed under running tap water to remove all soil and debris. The needed number of nematodes was extracted from the harvested roots by Sodium Hypochlorite Extraction Method (Hussey and Barker, 1973). The obtained nematode suspension was sieved through double sieves by 75-μm mesh on the top and 25-μm mesh opening on the bottom. Extracted eggs and J2s were collected into a flask and kept in a refrigerator for 4 ± 1 days at 4 °C for further applications.

Nematode inoculation

Four days after transplantation into the pots, bacteria-treated four-week-old tomato seedlings were inoculated with 1000 eggs or J2/pot. Inoculation was preceded by forming four holes with 2–3 cm apart around the plant root. Each hole was covered by soil following the application of evenly distributed nematode inoculum. The experiment was completed in 90 ± 2 days. Over the

course of the experimental period, all plants were irrigated regularly and fertilized with NPK (20-20-20) as needed.

Plant harvest

Ninety days after inoculation, the plants were cut off from crown part; the shoots were put in paper bags. Then, the plant roots were cleaned off from the soil by washing them gently by the tap water and keeping in the polyethylene bags in refrigerator for 4 ± 1 days at 4 °C for further processing.

Data collection

During plant growth, plants' heights were measured and recorded biweekly. At harvest, root and shoot fresh and dry weight were recorded. At harvest, root, fresh, and dry weight, shoot fresh, and shoot dry weight were recorded. Root-galling indices were also calculated by using a 0–5 scale (Taylor and Sasser, 1978). The number of egg masses was recorded after the roots were stained by food coloring for 2 h (Thies et al., 2002), based on a 0–5 scale (Taylor and Sasser, 1978).

Data analysis

The data of plant growth parameters as well as a root-galling index and egg-mass indices were subjected to analysis of variance (ANOVA), and means were separated ($P \leq 0.05$) by Duncan's multiple-range test using SPSS, version 20.0.0. The root-galling indices and egg-mass indices were transformed to $\log_{(x+1)}$ before analysis.

Results and discussion

The all bacterial treatments influenced ($P \leq 0.05$) all plant growth parameters as well as root-galling indices and egg-mass indices. There were significant differences of plant height among all treatments. The greatest plant height was recorded on the ZHA569-treated plants (59.03 ± 3.04 cm), while the lowest was in (+) control group (50.78 ± 3.70 cm). Also, the greatest root fresh weight was measured in ZHA90 (40.4 ± 9.49 g), and the lowest was in control (–) (21.1 ± 7.53 g). The greatest root dry weight was observed in ZHA90 (6.05 ± 2.35 g)-treated plants (Table 2).

In general, the greatest and the lowest values for shoot fresh weights were recorded in the controls (–) (111.10 ± 25.37 g) and (+) (54.70 ± 10.78 g). While the highest shoot dry weight was observed in control (–) (18.29 ± 4.52 g), the lowest was in the strain ZHA569 (11.96 ± 3.74 g) (Table 3).

Root-galling indices among treatments varied. However, the lowest root-galling indices were 0.678 ± 0.058 and 0.697 ± 0.042 for the strains ZHA296 and ZHA178, respectively. The lowest egg-mass indices were recorded at 0.727 ± 0.073 and 0.729 ± 0.059 for the strains ZHA296 and ZHA178, respectively (Table 3).

Table 2 Comparison of means of plant height, root fresh weight, and root dry weight of *Meloidogyne incognita*-inoculated tomato plants, following bacterial treatments (mean ± SD)

Treatments	Plant height (cm)	Root fresh weight (g)	Root dry weight (g)
Control +	50.78 ± 3.70d	30.2 ± 11.5bcd	4.45 ± 1.95a–d
Control –	56.23 ± 3.31abc	21.1 ± 7.53e	2.78 ± 1.52d
ZHA246	55.55 ± 6.54abc	31.6 ± 9.57a–d	3.46 ± 1.55 cd
ZHA235	54.83 ± 5.77a–d	35.8 ± 8.23a–d	3.90 ± 1.57 cd
ZHA90	56.45 ± 4.70abc	40.4 ± 9.49a	6.05 ± 2.35a
ZHA17	57.68 ± 4.36ab	36.8 ± 10.02a–d	4.93 ± 2.08abc
ZHA212	53.03 ± 4.10bcd	33.4 ± 3.47abcd	3.98 ± 0.94 cd
ZHA215	55.93 ± 3.29abc	34.6 ± 5.10a–d	3.58 ± 0.87 cd
ZHA287	56.10 ± 4.02abc	33.5 ± 9.01a–d	3.96 ± 1.51 cd
ZHA579	56.32 ± 5.68abc	37.5 ± 11.86abc	5.67 ± 1.25ab
ZHA191	54.83 ± 3.54a–d	27.3 ± 10.56de	3.16 ± 1.54 cd
ZHA308	55.33 ± 5.20a–d	39.5 ± 8.45ab	5.70 ± 2.19ab
ZHA178	52.30 ± 4.85 cd	34.7 ± 8.15a–d	4.40 ± 1.23a–d
ZHA57	56.33 ± 3.58abc	31.6 ± 9.50a–d	3.57 ± 1.17 cd
ZHA569	59.03 ± 3.04a	28.0 ± 10.55 cde	3.81 ± 2.29 cd
ZHA296	54.42 ± 6.20a–d	28.9 ± 9.35cde	3.71 ± 2.07 cd
ZHA88	56.75 ± 3.48abc	32.6 ± 6.54a–d	4.08 ± 1.24bcd

Data are means of 10 replications; means, followed by the same letter within a column, are not different according to Duncan's multiple-range test ($P \leq 0.05$)

Different microorganisms such as endophytic bacteria and fungi could be utilized in biological control arena to protect plants against soil borne pathogens. The endophytic bacteria and fungi are able to colonize the rhizosphere zone and plant endorhiza and, consequently, can promote plant health against root-knot nematodes (Sikora et al., 2007). Plant growth-promoting bacteria could enhance plant growth and nutrition, therefore increasing plant resistance against pathogens (Compant et al, 2005 and Liu et al., 2012).

In the current study, *Bacillus pumilus* strain ZHA90 increased plant height, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight and reduced root-galling numbers. However, this strain did not affect egg-mass numbers. These results are aligned with those reported by Lee and Kim (2016) for *M. arenaria* on tomato. Another study by Mekete et al. (2009) revealed that *B. pumilus* reduced root-galling and egg-mass numbers of *M. incognita* on Ethiopian coffee. *Bacillus megaterium* reduced egg hatching and the number of second-stage juvenile (J2) of *Meloidogyne incognita* (Huang et al., 2010), which was likely resulted from nematotoxic compounds or extracellular hydrolytic enzymes of bacteria destroying nematode eggshell and juvenile cuticle.

In our study, ZHA296 (*Paenibacillus castaneae*) significantly affected egg-mass and root-galling indices and shoot dry weight. In another study, *Paenibacillus* spp. reduced the rate of egg hatching and J2 number

Table 3 Comparison of means of shoot fresh weight, shoot dry weight, root-galling indices and egg-mass indices of *Meloidogyne incognita*-inoculated tomato plants, following bacterial treatments (mean ± SD)

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Root galling indices[a]	Egg mass indices[a]
Control +	54.70 ± 10.78d	12.92 ± 3.47 cd	0.778 ± 0.000a	0.778 ± 0.000a
Control −	111.10 ± 25.37a	18.29 ± 4.52a	0.000 ± 0.000d	0.000 ± 0.000c
ZHA246	63.40 ± 15.61 cd	13.91 ± 2.01bcd	0.762 ± 0.033ab	0.754 ± 0.038ab
ZHA235	60.30 ± 16.73d	13.60 ± 3.34bcd	0.754 ± 0.038ab	0.737 ± 0.060ab
ZHA90	91.00 ± 19.56b	15.85 ± 1.63b	0.739 ± 0.042b	0.739 ± 0.042ab
ZHA17	78.90 ± 23.35bc	14.44 ± 1.09bc	0.754 ± 0.038ab	0.745 ± 0.060ab
ZHA212	64.90 ± 8.85 cd	13.94 ± 1.67bcd	0.762 ± 0.033ab	0.762 ± 0.033ab
ZHA215	66.70 ± 9.68 cd	14.00 ± 1.15bcd	0.778 ± 0.000a	0.762 ± 0.033ab
ZHA287	67.10 ± 11.45 cd	13.75 ± 1.62bcd	0.770 ± 0.025ab	0.770 ± 0.025ab
ZHA579	70.50 ± 20.14 cd	14.48 ± 1.15bc	0.737 ± 0.060b	0.737 ± 0.060ab
ZHA191	72.80 ± 24.24 cd	13.94 ± 1.43bcd	0.754 ± 0.038ab	0.747 ± 0.041ab
ZHA308	59.30 ± 11.94d	13.17 ± 1.46 cd	0.778 ± 0.000a	0.778 ± 0.000a
ZHA178	58.40 ± 15.29d	13.36 ± 1.01 cd	0.697 ± 0.042c	0.729 ± 0.059b
ZHA57	57.50 ± 13.93d	12.95 ± 1.27 cd	0.754 ± 0.038ab	0.761 ± 0.056ab
ZHA569	65.40 ± 19.64 cd	11.96 ± 3.74d	0.762 ± 0.033ab	0.778 ± 0.000a
ZHA296	71.80 ± 20.56 cd	15.90 ± 2.26b	0.678 ± 0.058c	0.727 ± 0.073b
ZHA88	63.20 ± 10.49 cd	13.75 ± 1.48bcd	0.770 ± 0.025ab	0.770 ± 0.025ab

Data are means of 10 replications; means, followed with the same letter within a column, are not different according to Duncan's multiple-range test ($P \leq 0.05$)
[a]Root-galling and egg-mass indices: 0–5 scale, where 0 = no root galling or egg mass, 1 = 1–2 root galling or egg mass, 2 = 3–10 root galling or egg mass, 3 = 11–30 root galling or egg mass, 4 = 31–100 root galling or egg mass, and 5 = > 100 root galling or egg mass per root system

of *M. javanica*, *M. hapla*, *M. incognita*, *M. enterolobii*, *M. chitwoodi*, and *M. fallax*in vitro (Bakengesa, 2016). These results may be attributed to bacterial products such as antibiotics and secondary metabolites (Timmusk et al., 2005). Similarly, the study of Son et al. (2009) showed that among 40 strains of *Paenibacillus* spp. screened, *P. polymyxa* GBR-462 and GBR-508 and *P. lentimorbus* GBR-158 showed the strongest nematicidal activities and prevented *M. incognita* eggs from hatching. It can be elaborated that gelatinase and chitinase enzymes may contribute to the nematode inhibition (Jung et al., 2002).

Strain ZHA215 increased plant height, and strain ZHA178 reduced egg-mass and root-galling numbers significantly. Strain ZHA178 did not affect plant height, shoot dry weight, shoot fresh weight, root fresh weight, and root dry weight compared to control (+). It was reported that *Paenibacillus* spp. could cause phytotoxicity on plants depending on the concentration of bacteria and climatic conditions (Bakengesa, 2016).

Mycobacterium confluentis strain ZHA246 increased plant height significantly but did not affect other parameters. *M. immunogenum* strain ZHA17 increased plant height, shoot fresh weight, and shoot dry weight but did not affect root fresh weight, root dry weight, root-galling indices, and egg-mass indices. *Tsukamurella paurometabola* strain ZHA569 enhanced plant height but did not affect other parameters.

Conclusions

The soil contains plenty of beneficial bacteria, fungi, and other symbiotic organisms around plant roots. Bacteria can colonize in the rhizosphere zone or inner part of plant tissues and enhance plant growth resulting insignificant resistance to diseases by improving plant nutrition. In our study, ZHA90 (*B. pumilus*) increased plant growth and reduced root gall in tomato, and ZHA296 and ZHA178 (*P. castaneae*) decreased gall number and egg-mass numbers. On the other hand, ZHA17 (*M. immunogenum*) increased plant growth but not affected nematode-related parameters. The level of effectiveness of these strains on related parameters was significantly greater than other strains tested. These results suggest that *Bacillus pumilus*, *Paenibacillus costume*, and *Mycobacterium immunogenum* may be fairly good factors in suppressing the population density of *M. Incognito* tomato, despite of some discrepancy in the number of egg masses and the amount of root galling on the roots. These results also warrant additional long-term experiments with extended time to understand the dynamics of PGPR in field soil and to determine whether nematode population densities can be maintained at acceptable levels. Nevertheless, the results indicated that among 15 bacterial strains tested, ZHA296 and ZHA178 of *P. castaneae* and ZHA17 and ZHA57 of *M. immunogenum* could be used as promising biocontrol agents for the future nematode management strategies.

Acknowledgements
This study was funded by the Kahramanmaras Sutcu Imam University, BAP, project no: 2017/1-11 YLS.

Authors' contributions
All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Received: 16 June 2017 Accepted: 6 December 2017
Published online: 30 January 2018

References

Abad P, Favery B, Rosso MN, Castagnone-Sereno P (2003) Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol Plant Pathol 4:217–224

Bakengesa, J.A. 2016. Potential of *Paenibacillus* spp. as a biocontrol agent for root-knot nematodes (*Meloidogyne* spp.). Thesis in Master of Science in Nematology, Univ. of Gent, 31 pp.

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959

Hallmann J (2001) Plant interactions with endophytic bacteria. In: Jeger MJ, Spence NJ (eds) Biotic interactions in plant-pathogen associations. CABI pubs, New York, pp 87–120

Huang Y, Xu C, Ma L, Zhang K, Duan C, Mo M (2010) Characterization of volatiles produced from *Bacillus megaterium* YFM3. 25 and their nematicidal activity against *Meloidogyne incognita*. Eur J Plant Pathol 126(3):417–422

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis Rep 57:1025–1028

Hussey, R.S. and G.J.W. Janssen 2002. Root-knot nematode: *Meloidogyne* species. In: Resistance to parasitic nematodes: history, current use and future potential. Starr, J. L., R. Cook and J. Bridge, (Eds), pp. 43-70. CAB International, Wallingford

Insunza V, Alstrom S, Eriksson KB (2002) Root bacteria from nematicidal plants and their biocontrol potential against trichodorid nematodes in potato. Plant Soil 241(2):271–278

Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Manzanilla López R, Palomares Rius JE, Wesemael WML, Perry RN (2013) Top 10 plant parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14(9):946–961

Jung C, Wyss U (1999) New approaches to control plant parasitic nematodes. Appl Microbiol Biotechnol 51(4):439–446

Jung WJ, Jung SJ, An KN, Jin YL, Park RD, Kim KY, Shon BK, Kim TH (2002) Effect of chitinase-producing *Paenibacillus illinoisensis* KJA-424 on egg hatching of root-knot nematode (*Meloidogyne incognita*). J Microbiol Biotechnol 12(6): 865–871

Kloepper JW, Rodríguez-Kábana R, McInroy JA, Young RW (1992) Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: identification of fatty acid analysis and frequency of biological control activity. Plant Soil 139(1):75–84

Lee YS, Kim KY (2016) Antagonistic potential of *Bacillus pumilus* L1 against root-knot nematode, *Meloidogyne arenaria*. J Phytopathol 164(1):29–39

Liu J, Luo J, Ye H, Zeng X (2012) Preparation, antioxidant and antitumor activities in vitro of different derivatives of levan from endophytic bacterium, *Paenibacillus polymyxa* EJS-3. Food Chem Toxicol 50(3):767–772

Mekete T, Hallmann J, Kiewnick S, Sikora R (2009) Endophytic bacteria from Ethiopian coffee plants and their potential to antagonise *Meloidogyne incognita*. Nematology 11(1):117–127

Ralmi NHAA, Khandaker MM, Mat N (2016) Occurrence and control of root knot nematode in crops: a review. Aust J Crop Sci 11(12):1649–1654

Sikora RA (1988) Interrelationship between plant health promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Med Fac Landbouww Rijks Univ Gent 53(2b):867–878

Sikora RA, Fernández E (2005) Nematode parasites of vegetables. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CABI Publishing, Wallingford, pp 319–376

Sikora RA, Schäfer K, Dababat AA (2007) Modes of action associated with microbially induced in plant a suppression of plant-parasitic nematodes. Aust Plant Pathol 36(2):20–134

Son SH, Khan Z, Kim SG, Kim YH (2009) Plant growth-promoting rhizobacteria, *Paenibacillus polymyxa* and *Paenibacillus lentimorbus* suppress disease complex caused by root-knot nematode and *Fusarium* wilt fungus. J Appl Microbiol 107(2):524–532

Taylor, A.L. and J. N. Sasser 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne species*). Department of Plant Pathology, North Carolina State University, Raleigh

Thies JA, Merrill SB, Corley EL (2002) Red food colouring stain: new, safer procedures for staining nematodes in roots and egg masses on root surfaces. J Nematol 34:179–181

Tian B, Yang J, Zhang KQ (2007) Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbiol Ecol 61(2):197–213

Timmusk S, Grantcharova N, Wagner EGH (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl Environ Microbiol 71(11):7292–7300