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ORIGINAL ARTICLE

Biological control of the root-knot nematode *Meloidogyne incognita* on tomatoes and carrots by plant growth-promoting rhizobacteria

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Abstract

In the current study 27 plant growth-promoting rhizobacteria (PGPR) strains were screened for biocontrol activity against *Meloidogyne incognita* on 6-week old carrot seedlings that were inoculated with a suspension of J2 juveniles five days after treatment with the bacteria. Five of the PGPR strains, namely *Bacillus firmus* T11, *Bacillus aryabhattai* A08, *Paenibacillus barcinonensis* A10, *Paenibacillus alvei* T30, and *Bacillus cereus* N10w, caused 86.0, 85.2, 84.6, 81.5 and 82.1% reduction in gall numbers, respectively. In a subsequent greenhouse experiment on carrots, treatment with strain T30 caused a significant reduction in gall index and egg mass index compared to the control. The reduction in gall numbers caused by strain T30 was not statistically different from that obtained with CropGuard (furfural). Treatment with *B. aryabhattai* A08 gave the best results on tomato, significantly reducing gall index and egg mass index compared to the control. *In vitro* experiments with all five abovementioned strains resulted in second-stage juvenile (J2) paralyses. Induction of resistance was not observed in a split-root experiment conducted in the greenhouse, suggesting that secondary metabolites produced by the bacterial strains are responsible for the biocontrol activity. It is concluded that the bacterial strains *P. alvei* T30 and *B. aryabhattai* A08 have potential as biological control agents of *M. incognita* on carrots and tomatoes respectively.

Keywords *Bacillus* · Biocontrol · *Daucus carota* · *Paenibacillus* · PGPR · *Solanum lycopersicum*

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are some of the most destructive pests in agriculture and cause severe yield and economic losses in a wide variety of crops, including vegetables (Trudgill and Blok 2001; Kiewnick and Sikora 2006; Kalele et al. 2010; Collange et al. 2011). Carrots (*Daucus carota* L. subsp. *sativus* (Hoffm.) Schübl. and G. Martens) are also adversely affected worldwide since damage inflicted by root-knot nematodes infection causes severe losses in terms of yield and quality (Ajang 2010). Carrots play a major role in the supply of vitamin A and other nutrients in the human diet (Singh et al. 2012). Additionally, carrot is an important vegetable for the processing industry as well as the fresh market with more than 100,000 ton being produced annually in SouthAfrica (NDA 2014). Injury to the tap-root of the crop induces root abnormalities, such as forking and extensive galling.

Although carrot was the target crop in the current study, tomato (*Solanum lycopersicum* L.) was included as a model crop because of its fibrous root system, as opposed to the tap-root system of carrots. Furthermore, tomato is also severely affected by root-knot nematodes (Anwar and McKenry 2010) and is of major importance in South Africa and globally (Louw et al. 2006). Host plant resistance (De Almeida Engler et al. 2005) and synthetically-derived nematicides (Haydock et al. 2013) in particular, have been used for controlling root-knot nematodes. Concerns about the effects of these harsh chemicals on the environment and on human and animal health has led to a decrease in their use (Van der Putten et al. 2006), *e.g.* methyl bromide (Collange et al. 2011). This scenario gave impetus to the search for alternative measures to control root-knot nematodes.

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Rhizosphere-inhabiting bacteria, referred to as rhizobacteria or as plant growth-promoting rhizobacteria (PGPR), have shown considerable potential as biological control agents (Diby and Harshad 2014). It was shown that PGPR of the *Bacillus* genus reduced population densities of the soybean cyst nematode *Heterodera glycines* in greenhouse, microplot and field studies (Xiang et al. 2017). PGPR colonize the roots and rhizosphere of plants, with many of them producing metabolic by-products that affect either nematode motility, second-stage juvenile (J2) emergence and/or penetration (Siddiqui and Mahmood 1999). PGPR were previously shown to be effective against *Meloidogyne incognita* (Kofoid and White) Chitwood in *in vitro* studies and on various crops under field and greenhouse conditions (Ali et al. 2002; Akhtar and Siddiqui 2008; Terefe et al. 2009; Hashem and Abo-Elyousr 2011).

In the current study, trials were conducted to determine the potential of selected PGPR strains as biological control agents of the root-knot nematode *M. incognita* on carrots and tomatoes. A seedling bioassay was conducted on carrots to screen 27 PGPR strains for biocontrol activity. Subsequently, a greenhouse trial was conducted on both carrot and tomato plants with the five best performing strains selected from the seedling bioassay. The PGPR strains were assessed for their effect on root-knot nematode galling, reproduction and crop damage. Experiments were also conducted to study the possible mode of action of the selected bacterial strains. For this, possible systemic induced resistance by the bacteria was assessed by means of a split-root experiment on tomato. In addition, the direct effect of PGPR culture broth and culture filtrates on *M. incognita* J2 motility was studied.

Materials and methods

Nematode inoculum preparation

The *M. incognita* population used was maintained as a pure population in roots of susceptible tomato cv. Floradade plants in a greenhouse at the North-West University, Potchefstroom. The population was originally isolated from maize plants in Vryburg, North West. The identity of the population was confirmed by both morphological identification of female perineal-pattern morphology (Kleynhans 1991) and molecularly using the sequence characterized amplified region – polymerase chain reaction (SCAR-PCR) method (Zijlstra et al. 2000). Inoculum was obtained from these plants by extracting eggs from infected roots and hatching J2 according to an adapted NaOCl method (Riekert 1995).

PGPR inoculum preparation

PGPR strains were obtained from the Plant Pathology culture collection at the University of Pretoria. PGPR strains were revived from storage at −70 °C by streaking a single Microbank bead onto fresh nutrient agar and incubating the plates at 25 °C. Single colonies were picked up with a sterile inoculation loop and transferred into 100 mL sterile nutrient broth (Biolab) in 250 mL Erlenmeyer flasks. The cultures were then grown at 25 °C for 2 days in an incubator with shaking at 180 rpm. The culture broth containing bacteria at a concentration of 1×10^9 colony forming units per mL (cfu/mL) was used as inoculum in the seedling bioassay.

Seedling bioassay for screening of PGPR strains for nematode control on carrots

To evaluate the PGPR strains, a modification of the method described by Padgham and Sikora (2007) was used. Carrot cv. Dordogne seeds were planted in sterile seedling trays containing autoclaved vermiculite. Seedlings were grown in a greenhouse at 25–30 °C and were watered daily with sterile water. After 6 weeks, seedlings where transplanted to steam-sterilized soil in a sterilized plastic seedling tray (5 × 5 cm). Seedlings were inoculated with the PGPR strains (Table 1) by pipetting 1 mL of bacterial suspension (1×10^9 cfu/mL) to the soil around the base of each seedling at three sites separated 2 cm from each other, constituting a total of 3 mL per seedling. After 5 days, seedlings were inoculated with a suspension of 24-h old *M. incognita* J2 in sterile water around the base of each seedling at a rate of 1000 J2 per seedling. Each treatment was replicated five times, with one seedling per cell representing a replicate. Seedlings were kept in a greenhouse at temperatures ranging between 24.5 and 28.5 °C. Seedlings were watered with sterile water three times a week. Ten days after inoculation with the nematodes, seedlings were uprooted and evaluated for nematode infection by counting the number of galls present on the roots. The mean number of galls per gram of plant root was recorded and the percentage reduction of galls per plant calculated.

Biocontrol of root-knot nematode on carrot and tomato plants in the greenhouse

A greenhouse trial was conducted using the five best performing PGPR strains from the seedling bioassay. Seeds of carrot cv. Dordogne were planted in sterile polystyrene seedling trays containing autoclaved vermiculite. The seedlings were grown in a greenhouse at a temperature of 25–30.5 °C and watered daily with sterilized water. After 6 weeks, seedlings were replanted into 4 L plastic pots containing steam pasteurized soil. The same process was followed to obtain tomato cv. Moneymaker seedlings. Seedlings were then treated with 3 mL of bacterial culture broth per plant, by pipetting 1 mL into the soil at a 2-cm depth to ensure PGPR delivery to the root zone. CropGuard at 900 g (fufural active ingredient)/L (Illovo Ltd.) was included in the carrot experiment as a

Table 1 Effect of plant growth-promoting rhizobacteria (PGPR) strains on galling caused by *Meloidogyne incognita* on carrot roots under greenhouse conditions

Treatment	Galls/g roots (GR)	Mean GR rank*	GR rank	Percent gall reduction (%GR)	Mean %GR rank	%GR rank
T11	0.36	19.0	1	86.0	128.2	1
A10	0.38	21.9	2	84.6	119.9	2
A08	0.40	23.0	3	85.2	118.9	4
T30	0.43	24.4	4	81.5	119.3	3
N10w	0.47	32.7	5	82.1	108.9	5
T22	0.50	39.1	6	80.8	100.3	6
T18	0.75	42.3	7	71.2	97.6	7
A29	1.00	47.5	8	61.5	94.1	9
A07	1.25	58.4	9	51.9	85.8	10
A26Y	1.25	58.4	10	51.9	73.1	15
KBSIF3	1.33	63.6	11	48.8	71.0	17
A26W	1.57	64.1	12	39.6	76.5	14
T21	2.00	64.9	13	23.1	80.2	12
A40	1.45	66.6	14	44.1	77.3	13
A16	1.60	66.9	15	38.5	67.9	18
T26	1.57	70.1	16	40.0	84.9	11
T19	3.30	71.9	17	55.4	71.4	16
N30	1.71	72.3	18	34.2	64.5	19
T16	1.16	74.8	19	32.7	96.5	8
A32	1.64	82.7	20	37.1	60.2	20
T10	2.17	87.6	21	16.7	21.3	21
NAS6G6	2.50	87.6	22	2.7	45.9	23
T06	4.00	100.8	23	0	41.3	24
N19	4.00	104.5	24	0	38.4	25
A05b	4.25	115.1	25	0	26.7	27
T20	4.50	118.3	26	0	46.2	22
A06	6.00	130.3	27	0	36.0	26
*Mi*** only	2.57	–	–	–	–	–
Overall median = 1.4				Overall median = 52.8		
Chi-Square = 65.5				Chi-square = 70.2		
$p = 0.0002$				$p = 0.0001$		

*Mean rank according to Kruskall-Wallis test

***Meloidogyne incognita*

standard chemical treatment. However, this product was not included in the tomato experiment because it is not registered for use on this crop in South Africa.

Five days after the bacterial treatments were applied, the seedlings were inoculated with 1000 *M. incognita* J2, in the same manner as described for the carrot seedling bioassay. Each treatment was repeated five times, with one pot containing one plant representing a replicate. The temperature in the greenhouse ranged from 18.5 to 26.5 °C. The experiment was terminated 56 days after nematode inoculation. Plants were removed from each pot and the roots were washed under running tap water. For tomatoes shoot length was measured and root length was recorded for carrots. On both carrot and tomato plants, treatments with the PGPR strains were assessed for their effect on root-knot nematode galling, reproduction and subsequent crop damage. The number of galls on the roots were counted and the roots were then submerged in 0.05% Phloxine B solution for 20 min to facilitate staining of the egg masses. The use of root-knot nematode gall indices has shown to be a reliable, quick and easy method when determining nematode-plant interactions (Taylor and Sasser 1978; Dong et al. 2007 and Aalders et al. 2009). Thus, the following indices were used: (i) gall index 1 (GI-1) used for both carrots and tomatoes, where 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 galls (Taylor and Sasser 1978); (ii) gall index 2 used for carrots (GI-2C), where 0 = no galling, no forking, no stunting, marketable; 1 = 1 to 10 galls on secondary roots, taproot not affected, marketable; 2 =

11 to 50 galls, none coalesced, taproots with light forking, no stunting, unmarketable; 3 = 51 to 100 galls with some coalesced, forking, no stunting, unmarketable; 4 = more than 100 galls with some coalesced, severe forking and moderate stunting, unmarketable; 5 = more than 100 galls, mostly coalesced, severe stunting, unmarketable (Bélair and Parent 1996); (iii) gall index 2 used for tomato (GI-2T), where 0 = no galling; 1 = trace infection with a few small galls; 2 = 25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; and 5 > 75% roots galled (Hussey and Janssen 2002); (iv) egg mass counts per root system (stained with Phloxine) (EMI), as follows: 0 = no egg or galls, 1 = 1–2 eggs/galls, 2 = >2–11 eggs/galls, 3 = >11–30 eggs/galls, 4 = 31–100 eggs/galls and 5 = more than 100 eggs/galls (Taylor and Sasser 1978); and (v) Rf values based on number of eggs and J2 extracted and counted, where Rf = final population density (Pf)/initial population density (Pi) (Windham and Williams 1987). The greenhouse experiments were repeated once.

PGPR induced systemic resistance against root-knot nematode

A split-root system was used to determine whether selected PGPR strains A08, A10, T30, T11 and N10w were able to systemically induce resistance against *M. incognita*. Susceptible tomato cv. Moneymaker seeds were planted in seedling trays containing sterile vermiculite and maintained in a greenhouse at 26–29 °C. Seedlings were watered daily with heat-sterilized water. After 6 weeks, the root systems were excised, and incisions (4 cm in length) were made longitudinally such that the stems were split into two sides (Martinuz et al. 2012), which were re-planted into two separate but adjacent plastic trays (8 cm × 8 cm) containing a 1:1 steam-pasteurized soil: river sand mixture. Lower leaves were removed from the stem to prevent vegetative growth. Two weeks were allowed for new root systems to develop before inoculation with the PGPR strains. Plants were first inoculated with the bacterial strains on the inducer side of the root system. In each treatment with bacterial strains, roots on the inducer side were inoculated with 3 mL of a bacterial broth suspension of a specific isolate per pot. Inoculation was done by pipetting 1 mL of bacterial suspension (1×10^9 cfu/mL) at three locations around the base of each seedling. Roots on the responder side were not treated with PGPR. Sterile water was used to inoculate the inducer side of the control plants. After 14 days, the seedlings were inoculated on the responder side applying 4 mL of a suspension containing 1000 *M. incognita* J2 in sterile water, around the base of each seedling using a pipette. Each treatment was replicated six times, with one plant representing one replicate. Plastic trays were organized in a complete randomized block design (CRBD). The experiment was conducted twice. Ten days after nematode inoculation, plants were removed from the plastic trays, both the inducer and responder roots were excised, washed, and root-knot nematode galls were counted.

Effect of PGPR culture broth and cell-free filtrate on *M. incognita*

For the *in vitro* analysis, the method described by Terefe et al. (2009) was used. Bacterial cultures were prepared in nutrient broth as described earlier. Cell-free filtrates were prepared by pelleting the bacterial cells by centrifugation at 4000 rpm for 20 min. The supernatant was then passed through a 0.22 μm filter membrane and the flowthrough was used as a cell-free filtrate. To determine the effect of the culture filtrates on *M. incognita,* 5 mL were added to a plastic micro well together with 3 mL sterile water and 1 mL of a suspension containing 100 J2 in sterile water (total volume 9 mL). Each treatment was replicated 10 times. Sterile water was used as a negative control and 97% ethanol as the positive control. The micro plates were covered in plastic wrap to prevent evaporation and placed in an incubation chamber at 26 °C. After 3, 6, and 24 h (Andalo et al. 2012), J2 motility was observed using a stereomicroscope. Nematode immobility was confirmed by poking J2 with a needle. A second *in vitro* experiment was conducted as described above, except that 5 mL of bacterial culture broth (containing bacterial cells), instead of culture filtrate, were added to the micro wells.

Statistical analysis

For the screening bioassay, data (mean number of galls per gram plant root) were subjected to non-parametric analysis using the Kruskal-Wallis test. For the greenhouse experiment, since similar results were obtained for both experiments, data were pooled before analyses. Data regarding nematode infection and induced systemic resistance were subjected to ANOVA and Fisher LSD test. Data from the *in vitro* experiments (effect of bacterial culture broth and cell-free filtrate) were subjected to repeated measure ANOVA (MANOVA) and Tukey test. All statistical analyses were performed at a $p \leq 0.05$ using packages available at STATISTICA (www.Stasoft.com).

Results

Seedling bioassay for screening of PGPR that control root-knot nematode on carrots

No galls were detected on control seedlings treated with tap water only. Infection of carrot roots by *M. incognita* was confirmed since galls developed on plants non-treated with PGPR and inoculated with nematodes. Inoculation with PGPR strains T11, A10, A08, T30 and N10w resulted in the lowest number of galls formed per gram of roots (0.36–0.47 galls/g

roots), reducing gall formation by between 81.5 and 86.0% compared to the control without PGPR treatment (Table 1). An increase in galling was recorded on roots for seedlings treated with PGPR strains T06, N19, A05b, T20 and A06. The average number of galls per gram roots for all treatments was 1.4 galls/g, and the average percentage reduction of galls per gram roots for all treatments was 52.8%.

The *p* values in both evaluations were highly significant ($p = 0.002$ for galls per gram roots, and $p = 0.001$ for percentage gall reduction) indicating treatment with different PGPR strains reduced galling. Furthermore, treatments with the five best isolates (T11, A08, A10, T30 and N10w) differed from treatments with the other isolates. Treatment with each of these five PGPR resulted in lower galls per gram roots when compared to the median (1.4 galls/g) of all the treatments. The identity of these isolates was previously determined at the University of Pretoria by means of 16S-rRNA sequencing (Breedt et al. 2017) as *Paenibacillus barcinonensis* (A10), *Bacillus aryabhattai* (A08), *Paenibacillus alvei* (T30), *Bacillus firmus* (T11) and *Bacillus cereus* (N10w).

Biocontrol of root-knot nematode on carrot and tomato in the greenhouse

The presence of galls on carrot and tomato roots confirmed the pathogenicity of the *M. incognita* inoculum. Compared to the untreated control (only inoculated with *M. incognita* J2), treatment with bacterial strain T30 suppressed gall formation on carrot roots as reflected by GI-1 values (2.2 *vs.* 3.2 for control plants; Table 2). The GI-1 for plants treated with strain T30 was not statistically different from that of plants treated with CropGuard, which had the lowest GI-1 (1.4) and differed from those of all other treatments. Treatment of plants with strains A10, A08, T11, and N10w had no significant effect since the GI-1 values were similar to that of the control plants. Based on GI-2C assessments, treatment with strains T30, N10w, A10, T11 (1.4, 2.1, 2.2, and 2.3, respectively) and CropGuard (0.9), reduced galling on carrot roots compared to that of the untreated control (3.3) that was only inoculated with *M. incognita* J2 (Table 2).

According to EMI values, treatment with strains T30 (2.0) and T11 (2.1) reduced the number of *M. incognita* egg masses and J2 extracted from carrot roots compared to the number recovered from untreated controls that were only inoculated with *M. incognita* (3.1) (Table 2). However, these strains were not as effective in controlling the nematode as CropGuard, which had the lowest EMI (0.6).

Interestingly, no significant differences were recorded between the root lengths of plants subjected to the different treatments (Table 2). Although the chemical treatment showed the lowest values for the gall and egg mass indices, plants showed signs of phytotoxicity. None of the treatments had a Rf value less than 1.0, indicating that the Pi value of 1000 J2 and eggs was not reduced by any of the treatments during the evaluation period.

According to GI-1 values, treatment with strains A08 (3.7) and T11 (3.9) reduced galling on tomato roots compared to untreated control plants (4.7; only inoculated with *M. incognita* J2). Treatment with strains T30 (4.3), A10 (4.0) and N10w (4.5) did not result in any significant reduction in galling (Table 3). However, according to GI- 2T no treatment significantly reduced galling compared to the control plants (Table 3). Egg mass index was reduced from 4.6 in control plants (untreated but inoculated with *M. incognita*) to 3.9 and 3.6 in plants treated with strains A08 and T11, respectively (Table 3). None of the treatments had any significant effect on shoot length of tomato plants (Table 3). As for carrots, none of the treatments had a Rf of less than 1.0 which

Table 2 Effect of selected plant growth-promoting rhizobacteria (PGPR) strains on root length and galling on carrot seedlings infected by *Meloidogyne incognita* under greenhouse conditions

Treatment	Gall index 1 (GI-1; 1–5)	Gall index 2 (GI-2C; 1–5)	Egg mass index (EMI; 1–5)	Root length (cm)
T30	2.2 ab	1.4 ab	2.0 b	5.8 a
A10	2.7 bc	2.2 bc	2.3 bc	6.5 a
A08	3.2 cd	2.6 cd	3.0 c	6.1 a
T11	3.4 cd	2.3 bc	2.1 b	6.5 a
N10w	3.5 d	2.1 b	2.8 cd	5.9 a
CropGuard	1.4 a	0.9 a	0.6 a	5.8 a
Mi only	3.2 cd	3.3 d	3.1 e	6.0 a
p value	0.000	0.000	0.000	0.100

For each column, values are the means of two independent experiments; means with the same letter do not differ significantly according to Fisher's LSD test ($p \leq 0.05$)

Mi = *Meloidogyne incognita*

Table 3 Effect of selected plant growth-promoting rhizobacteria (PGPR) strains on galling and shoot length of tomato plants infected by *Meloidogyne incognita* under greenhouse conditions

Treatment	Gall index 1 (GI-1; 1–5)	Gall index 2 (GI-2T; 1–5)	Egg mass index (EMI; 1–5)	Shoot length (cm)
T30	4.3 abc	3.9 ab	4.3 ab	46.9 a
A10	4.0 abc	3.8 ab	4.3 ab	51.6 a
A08	3.7 a	3.6 a	3.9 bc	45.4 a
T11	3.9 ab	3.9 ab	3.6 c	44.9 a
N10w	4.5 bc	4.4 b	4.6 a	50.9 a
Mi only	4.7 c	4.0 ab	4.6 a	42.0 a
p value	0.025	0.068	0.025	0.100

For each column, values are the means of two independent experiments; means with the same letter do not differ significantly according to Fisher's LSD test ($p \leq 0.05$)

Mi = *Meloidogyne incognita*

indicates that the Pi value of 1000 J2 and eggs was not reduced by any of the treatments during the experiment.

PGPR do not induce systemic resistance against root-knot nematode

To test the potential of PGPR strains to induce systemic resistance, tomato roots were separated in a split-root system and PGPRs were applied to the inducer side and *M. incognita* J2 to the responder side. PGPR application to the inducer side had no significant effect on gall production in the responder side. Similar results were obtained for both experiments conducted (Table 4). Treatment with strain *P. barcinonensis* A10 resulted in the lowest number of galls present on the responder side of the root in both experiments with a mean reduction in gall numbers of 37.7%, but these numbers were not statistically different from those for the untreated control plants.

Effect of PGPR culture broth and cell-free filtrates on *M. incognita* motility

During the *in vitro* assays conducted in micro wells, culture filtrates of strains A10, N10w, A08, T11 and T30 caused a significant increase ($p \leq 0.05$) in J2 paralysis after 24 h exposure compared to the water control. The percentage of immotile J2 were 31, 52, 56, 59 and 79% for treatments with cell-free filtrates of bacterial strains A10, N10w, A08, T30 and T11, respectively. Exposing J2 to filtrates of strain T11 resulted in the highest number of paralyzed nematodes after 24 h. In the tests conducted with bacterial culture broth (containing bacterial cells), all PGPR treatments caused significant ($p \leq 0.05$) increases in J2 paralysis. Treatment with strains A10, N10w, T30, A08 and T11 resulted in 72, 94, 97, 97 and 98% J2 paralyzed after 24 h, respectively. As for the experiment with culture filtrates, strain T11 again resulted in the highest percentage of J2 paralyzed after 24 h exposure. All treatments resulted in a number of J2 paralyzed not significantly different from that for the 97% ethanol control after 24 h exposure. None of the bacterial treatments equaled the level of paralysis induced by 97% ethanol during the 3 and 6 h exposures in both experiments. All the bacterial culture broth treatments caused a significantly higher percentage of J2 paralysis in comparison with the water control (negative control) at all-time intervals (Table 5). There was a tendency for slightly more J2 paralysis when bacterial culture broth was used compared to cell-free filtrate.

Table 5 Percent paralyzed *Meloidogyne incognita* second-stage juveniles (J2) caused by broth cultures and culture filtrates of selected plant growth-promoting rhizobacteria (PGPR) strains in *in vitro* bioassays

Treatment	Percent paralized J2 Cell-free filtrate	Percent paralized J2 Culture broth
A10	17.9 b	26.7 b
T11	38.0 e	38.0 b
N10w	25.9 c	33.6 b
A08	30.7 cd	34.4 b
T30	32.3 d	36.5 b
Sterile water	4.8 a	10.5 a
97% ethanol	100.0 f	99.6 c
p value	0.000	0.000

Data indicate mean percent paralyzed J2 values pooled for time intervals 3, 6 and 24 h. Means in the same column followed by same letter do not differ significantly according to Tukey HSD test ($p \leq 0.05$)

Discussion

The ability of PGPRs to act as biological control agents against the root-knot nematode *M. incognita* was confirmed as a result of this study. These results clearly indicate the biocontrol potential of these bacterial strains against *M. incognita* and are in agreement with numerous other studies (Keren-Zur et al. 2000; Siddiqui et al. 2011; Hallmann et al. 2009; Xiang et al. 2017) that reported biological control potential of PGPR strains against plant-parasitic nematodes, including *Meloidogyne* spp.

The negative results obtained in the split-root experiment and the positive results obtained in the *in vitro* assay suggest

Table 4 Effect of treatment with selected plant growth-promoting rhizobacteria (PGPR) strains on the number of galls caused by *Meloidogyne incognita* on responder roots of tomato plants grown in a split-root system in the greenhouse

Treatment	Number of galls Experiment 1	Number of galls Experiment 2	Mean number of galls for two experiments	Percent gall reduction*
A10	7.5 b	6.0 a	6.8 a	37.7
T11	11.4 ab	9.3 a	10.4 ab	3.6
N10w	14.6 ab	11.0 ab	10.8 ab	0.0
A08	17.0 a	12.3 ab	14. 7 ac	0.0
T30	16.4 a	18.8 b	17.6 c	0.0
Mi only	11.0 ab	10.7 ab	10.8 ab	–
p value	0.088	0.061	0.006	–

Means in the same column with the same letter do not differ significantly according to Fisher's LSD test ($p \leq 0.05$)

*Mi = *Meloidogyne incognita*, inoculated only on the responder side of the seedlings

that extracellular toxic compounds or enzymes rather than induced resistance is involved in the mode of action of the effective PGPR strains. Our results concur with many similar findings as it is widely reported that *Bacillus* and *Paenibacillus* species produce toxic compounds that are inhibitory to the growth and activities of nematodes. For example, Tong-Jian et al. (2013) reported that a cell-free filtrate of *B. cereus* increased mortality of *M. javanica* J2, suggesting that extracellular nematicidal substances were present in the filtrate. Similarly, Lian et al. (2007) reported on *Bacillus* spp. producing nematicidal and cuticle-degrading extracellular molecules, such as serine alkaline protease, that reduced nematode action. *Bacillus cereus* was further shown by Huang et al. (2016) to lower J2 hatching rates and cause higher mortality of J2 than the untreated control *in vitro*, suggesting it to be due to extracellular nematicidal substances. Also, direct nematode antagonism of other *Bacillus* of known (e.g. *B. cereus*) and unknown species (Gardener 2004) has been reported. Nematicidal activities for *Paenibacillus* spp. have also been reported. Exposure of *M. incognita* to a culture filtrate of *P. polymyxa* had a significant effect on J2 hatching, J2 mortality, root galling and overall nematode population densities under *in vitro* conditions (Chauhan et al. 2015). Other mechanisms of biocontrol by *Bacillus* spp. include interfering with recognition, production of toxins, nutrient competition and induced systemic resistance (Tian et al. 2006).

Production of antibiotics could also be responsible for reducing root-knot nematode activity. *Bacillus* spp. are reported to produce phenazines (Gardener 2004) which, at a concentration of 50%, have been shown to cause mortality rates of 92.5% of *M. incognita in vitro* (Sankari et al. 2013). Furthermore, *B. thuringiensis* is known to produce crystalline proteins called delta-endotoxins (Jisha et al. 2013), which can be disruptive to the digestive tracks of nematodes by forming pores in the membranes (Berlitz et al. 2013).

PGPR have increasingly been commercialized as both biocontrol agents and biofertilizers (Saharan and Nehra 2011). This is mainly due to their ability to suppress diseases, improve plant nutrient acquisition, produce phytohormones, induce systemic resistance, produce siderophores and inflict antibiosis (Saharan and Nehra 2011). Internationally, products containing PGPR have been registered for control of root-knot nematodes and currently include the products BioNem, Nortica and Votivo (all containing *B. firmus*), Econem (*Pasteuria penetrans*), and Clariva (*Pasteuria nishizawae*), Deny and Blue circle (*Burkholderia cepacia*), Biostart (*Bacillus* spp. mixture), Bio Yield (*P. macerans* and *B. amyloliquefaciens*) and Nemix (*Bacillus* spp.) (Lamovšek et al. 2013; Tian et al. 2006). The product Bioarc has shown the ability to control root-knot nematodes, and has been deemed a viable alternative to nematicides (Radwan et al. 2012). Similarly, Terefe et al. (2009) reported reduced number of eggs, galls and final nematode populations in tomato plants treated with the product Bionem (containing *B. firmus* as the active ingredient) under greenhouse and field conditions. The availability of commercial biological products and the further development of such products advances the eco-friendly control of the root-knot nematode *M. incognita*. The results of the current study indicate that the strains *B. cereus* N10w, *B. aryabhattai* A08, *B. firmus* T11, *P. alvei* T30 and *P. barcinonensis* A10 have potential to be further developed as biocontrol agents of root-knot nematodes.

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