INDUCED SYSTEMIC RESISTANCE OF SELECTED ENDOPHYTIC BACTERIA AGAINST *MELOIDOGYNE INCOGNITA* ON TOMATO

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SUMMARY

In previous work, the four endophytic bacteria *Pantoea agglomerans* MK-29, *Cedeca davisae* MK-30, *Enterobacter* spp. MK-42 and *Pseudomonas putida* MT-19 were shown to reduce *Meloidogyne incognita* on tomato when applied as a seed treatment and/or soil drench. The objective of this work was to study these bacteria for their potential to induce systemic resistance against root knot nematodes on tomato. To guarantee spatial separation between inducing agent and pathogen a split-root system was chosen and inoculated with the bacteria as a drench application on one side of the root system and 6 days later with 2000 juveniles of *Meloidogyne incognita* on the other side of the split-root system. The experiment was maintained in the greenhouse and repeated once. The penetration rate of juveniles as well as the total number of root-knot galls and egg masses was recorded. Treatment with all four bacteria significantly reduced juvenile penetration and the number of root-knot galls when compared with the non-treated control. Induced systemic resistance is considered a possible control mechanism of endophytic bacteria against root-knot nematodes.

INTRODUCTION

Endophytic bacteria are ubiquitous to most plant species, and internal colonizers of the plant that do not harm the plant (Hallmann *et al.*, 1997a). Some of these endophytic bacteria are beneficial to the plant, i.e. they stimulate plant growth and health. Once inside the plant, endophytic bacteria can colonize potential infection sites of plant pathogens. The plant environment also protects the endophytes against environmental stresses. For these reasons, there is considerable interest in endophytic bacteria as biocontrol agents especially those providing economical features such as plant growth promotion and stimulation of plant defence mechanisms (Hallmann, 2001). Previous reports have shown that bacterial endophytes reduce disease symptoms caused by plant pathogens such as *Fusarium oxysporum* f. sp. *vasinfectum* on cotton (Chen *et al.*, 1995), *Rhizoctonia solani* on potato and cotton (Pleban *et al.*, 1995) and *Meloidogyne incognita* on tomato and cucumber (Hallmann *et al.*, 1997b; Munif *et al.*, 2000).

The mechanisms of how endophytic bacteria stimulate plant growth and health are poorly studied, but might be similar to those described for plant growth-promoting rhizobacteria (Höflich *et al.*, 1994; Kloepper *et al.*, 1991). Potential mechanisms include: 1) direct antagonism, 2) niche exclusion, 3)

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competition, 4) induction of systemic resistance (ISR), and 5) enhanced plant tolerance towards biotic and abiotic stresses (Hallmann, 2001). CHEN *et al.* (1995) assumed that the biocontrol effect of some endophytic bacteria may be a result of enhanced host defense rather than from bacterial metabolites favoring ISR as potential mechanism. Based on Kloepper *et al.* (1992) "induced disease resistance is the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents". The objective of the present study was to test four endophytic bacteria with known antagonism towards *M. incognita* for their potential to induce systemic resistance on tomato.

MATERIALS AND METHODS

Bacterial inoculum. The endophytic bacteria used in this study were originally isolated from tomato roots. The bacteria were identified based on fatty acid analysis (FAME-GC) as *Pantoea agglomerans* MK-29, *Cedecea davisae* MK-30, *Enterobacter* spp. MK-42 and *Pseudomonas putida* MT-19. The bacteria were cultured in tryptic soy broth (TSB) under continuous shaking on a rotary shaker at 100 rpm for 36 h at 24°C. The bacterial suspension was centrifugated at 4600 g for 20 min, and the bacterial pellet was resuspended in sterile ¼-strength Ringer's solution. The bacterial suspension was then adjusted to OD_{560} = 2.0 (approx. 10^{10} cfu ml^{-1}).

Nematode inoculum. *Meloidogyne incognita* (Kofoid & White) Chitwood, race 3, was used as nematode inoculum. Nematode eggs were extracted from 3 month-old galled tomato roots following the technique described by Hussey and Barker (1973). The roots were macerated in a Warring blender for 20 sec, transferred into a flask containing 500 ml of a 1.5 % NaOCl solution and shaken for 3 min to free eggs from the gelatinous matrix. The egg suspension was then rinsed over a sieve combination of 100 µm and 25 µm mesh size to remove excess chlorine. Eggs collected on the 25 µm-aperture sieve were transferred into tap water and agitated for 10 days at 24°C to stimulate juvenile hatching. The juveniles were separated from the eggs using a modified Oostenbrink dish. The number of juveniles was adjusted to 1000 juveniles/ml and 2 ml were used as inoculum in the experiments.

Split root system. Tomato plants were grown in a three-pot-system with each pot measuring 9 cm in diameter. Three week-old tomatoes cv. Hellfrucht Frühstamm were transplanted into the upper pot. Two wide openings in the bottom of the upper pot allowed the roots to equally grow into the two lower pots to result in a split-root system with two spatially seperated root systems. Previous experiments have shown, that no bacterial growth occur from one side of the root system to the other occurs (Munif, pers. communication).

Experiment 1. In this experiment the presence of bacteria-mediated ISR toward early root peneration of *M. incognita* was studied. Two weeks after transplanting the tomato roots had colonized the lower pots. One side of the split-root system was inoculated with 5 ml of the bacterial suspension and 6 days later, the other side of the split-root system, was inoculated with 2000 juveniles of *M. incognita*. Control plants were inoculated with 5 ml ¼-strength Ringer's solution to one side and 2 ml tap water to the other side of the split-root system. Twelve days after nematode inoculation, the experiment was terminated. The root fresh weight of both root halves was measured and the penetration rate of juveniles into the root system was recorded. For the latter, the roots were stained in a 0.01% acid fuchsin solution. The experiment consisted of a control and the four endophytic bacteria described above with and without *M. incognita* inoculation. Each treatment was replicated 6 times.

Experiment 2. The experiment was arranged in the same manner as experiment 1 except that it was terminated 6 weeks after nematode inoculation. Root fresh weight and the number of galls and egg masses was measured for both sides of the split-root system.

Data were analyzed according to standard analysis of variance procedures with the software Statgraphics (Statistical Graphics Corp., Rockville, MD). Duncan's multiple range test was used for mean comparison. Statistical differences referred to in the text were significant at $P = 0.05$.

RESULTS

The application of endophytic bacteria to one side of a split-root system and *M. incognita* to the other side resulted in a significant reduction in juvenile penetration compared with the control (Figure 1). The highest reduction in juvenile penetration was achieved for *P. putida* MT-19 (67 %) followed by *P. agglomerans* MK-29 (61 %) and MK-30 (54 %). Similarly, the number of galls (Figure 2) and egg masses (Figure 3) caused by *M. incognita* were significantly reduced in tomato treated with endophytic bacteria. The number of galls was reduced from 29% for *Enterobacter* spp. MK-42 to 37% for *P. putida* MT-19, while the reduction in the number of egg masses was reduced up to 56% for *P. putida* MT-19. The root fresh weight was lower on the nematode-inoculated side of the split-root system than on the nematode-free side (Figure 4). Differences in the root fresh weight between treatments were not significant.

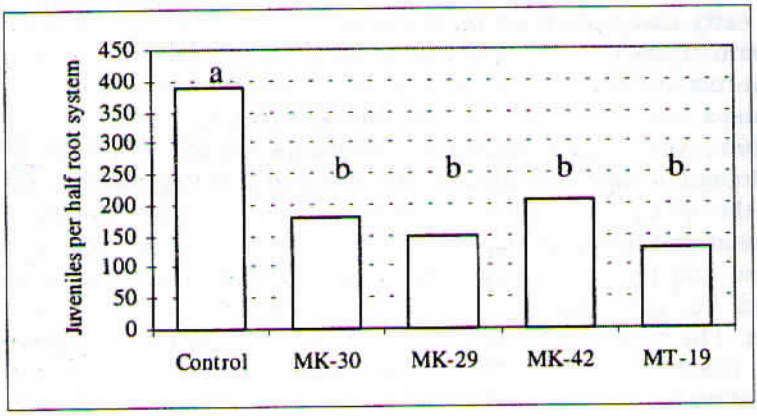

Figure 1: Effect of the endophytic bacteria *Pantoea agglomerans* MK-29, *Cedecea davisae* MK-30, *Enterobacter* spp. MK-42 and *Pseudomonas putida* MT-19 on the penetration rate of *Meloidogyne incognita* juveniles into the non-bacterized side of a tomato split-root system. Means with the same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$, n = 6).

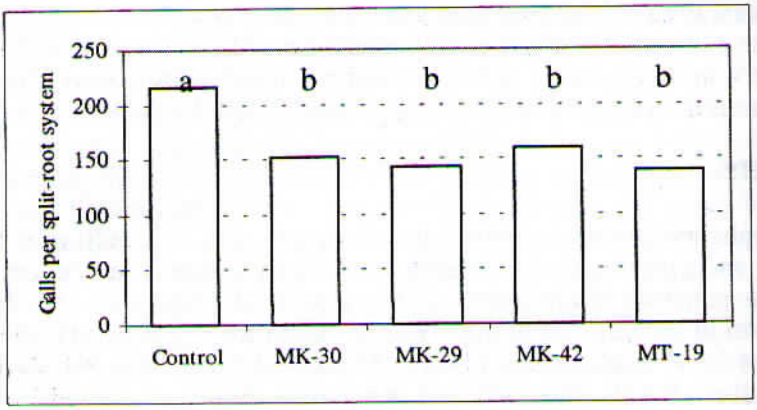

Figure 2: Effect of the endophytic bacteria *Pantoea agglomerans* MK-29, *Cedecea davisae* MK-30, *Enterobacter* spp. MK-42 and *Pseudomonas putida* MT-19 on the number of galls caused by *Meloidogyne incognita* juveniles on the non-bacterized side of a tomato split-root system. Means with the same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$, n = 6).

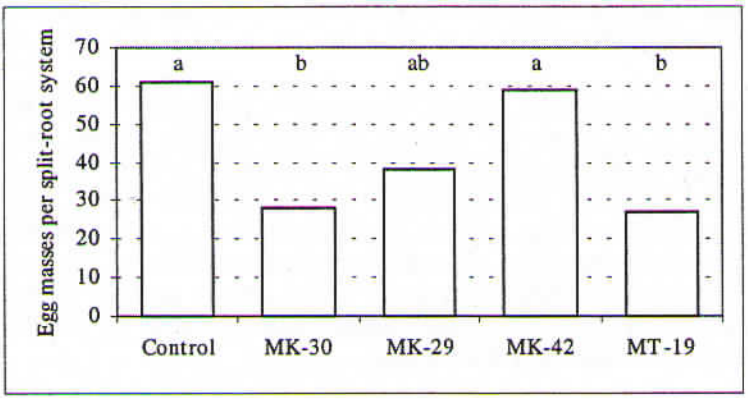

Figure 3: Effect of the endophytic bacteria *Pantoea agglomerans* MK-29, *Cedecea davisae* MK-30, *Enterobacter* spp. MK-42 and *Pseudomonas putida* MT-19 on the number of egg masses caused by *Meloidogyne incognita* juveniles on the non-bacterized side of a tomato split-root system. Means with the same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$, n = 6).

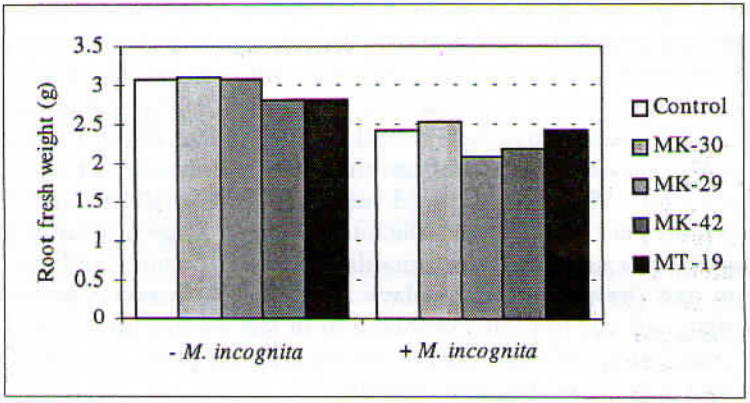

Figure 4: Effect of the endophytic bacteria *Pantoea agglomerans* MK-29, *Cedecea davisae* MK-30, *Enterobacter* spp. MK-42 and *Pseudomonas putida* MT-19 on the root fresh weight of a tomatoes split-root system. The differences between treatments were not significantly different based on Duncan's multiple range test ($P < 0.05$, n = 6).

DISCUSSION

Previous studies have shown that rhizobacteria-mediated induced systemic resistance (ISR) protects the plant against a broad spectrum of viral, bacterial and fungal pathogens as well as insects (van Loon *et al.*, 1998; Wei *et al.*, 1996; Zehnder *et al.*, 1999). Rhizobacteria-mediated ISR against plant parasitic nematodes has been reported by Hasky-Günther and Sikora

(1995) and Martinez-Ochoa *et al.* (1995) for *Globodera pallida* and *M. i*
cognita, respectively.

In the present study, the endophytic bacteria *P. agglomerans* MK-29, *(*
davisae MK-30, *Enterobacter* spp. MK-42 and *P. putida* MT-19, that prev
ously demonstrated biocontrol potential against *M. incognita*, were teste
for ISR as a potential mode-of-action. The application of the endophyti
bacteria to one side of the split-root system resulted in a significant reduc
tion of juvenile penetration as well as number of galls and egg masses o
the other side of the split-root system. These results strongly indicate IS
as control mechanism and characterize these bacteria as plant healt
promoting rhizobacteria (Sikora, 1988).

Although ISR was demonstrated, the origin of the inducing agent is sti
unknown. In general, ISR can be caused by microbial metabolites and/o
bacterial cell constituents. Hasky-Günther *et al.* (1998) and Reitz *et a*
(2000) studying the inducing agent of the rhizobacteria *Rhizobium etli* G1
and *Bacillus sphaericus* B43 causing ISR in potato towards the potato cys
nematode *G. pallida*, found that both viable and heat-killed cells of *R. et*
G12 and *B. sphaericus* B43 significantly reduced nematode penetration
Conversely only the culture filtrates of *B. sphaericus* B43 and not of *R. et*
G12 induced systemic resistance indicating different modes-of-action. Fo
R. etli G12 it was shown that purified bacterial lipopolysaccharides (LPS
but not exopolysaccharides (EPS) acted as the inducer of the plant defens
response. Similar LPS extracted from the outer membrane of *Pseudomona*
fluorescens strain WCS417r induced resistance in carnations against Fusa
rium wilt (van Peer *et al.*, 1991). Bacterial LPS are known to bind to plan
cells (Costerton *et al.*, 1987), thus enabling signal transmission between the
bacterium and the plant. If LPS plays a key role in bacteria-mediated in
duced resistance, endophytic colonization of the bacterium could improv
signal transmission between the bacterium and the plant thus leading to a
stronger and more persistent ISR response.

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