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CONTENTS

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

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

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

REFERENCES

Chen, C., Bauske, E.M., Musson, G., Rodríguez-Kábana, R. and Kloepper, J.W. (1995). Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biol. Control 5, 83-90.

Costerton, J.W., Cheng, K.J., Geesy, G.G., Ladd, J.C., Nickel, J.C., Dasgupta, M. and Marrie, T.J. (1987). Bacterial biofilms in nature and diseases. Ann. Rev. Microbiol. 41, 435-464.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper, J.W. (1997a). Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43, 895-914.

Hallmann, J., Rodríguez-Kábana, R. and Kloepper, J.W. (1997b). Nematode interactions with endophytic bacteria. In: A. Ogoshi, K. Kobayashi, K. Homma, F. Kodama, N. Kondo and S. Akino (Eds.). Plant Growth-Promoting Rhizobacteria, Present Status and Future Prospects. Proc. 4th Intern. Workshop on Plant Growth-Promoting Rhizobacteria. OECD Joint Workshop, Sapporo, Japan, pp. 243-245.

Hallmann, J. (2001). Plant interactions with endophytic bacteria. In: M.J. Jeger and N.J. Spence (Eds.). Biotic Interaction in Plant-Pathogen Association. CABI Wallingford, UK (in press).

Hasky-Günther K. & Sikora R.A. (1995). Induced resistance mechanism induced systemically throughout the root system by rhizosphere bacteria towards the potato cyst nematode *Globodera pallida*. Nematologica 41, 306

Hasky-Günther K., Hoffmann-Hergarten S. & Sikora R.A. (1998). Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). Fundament. Appl. Nematol. 21, 511-517.

Höflich, G., Wiehe, W. & Kühn, G. (1994). Plant growth stimulation by inoculation with symbiotic and associative rhizosphere microorganisms. Experientia 59, 897-905.

Hussey, R.S. and Barker, K.R. (1973). A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57: 1025-1028.

Kloepper, J.W., Zablotowitz, R.M., Tipping, E.M. and Lifshitz, R. (1991). Plant growth promotion mediated by bacterial rhizosphere colonizers. In: D.L. Keister & P.B. Cregan (Eds.). The Rhizosphere and Plant Growth. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 315-326.

Kloepper, J.W., Tuzun, S. and Kuc, J. (1992). Proposed definition related to induced disease resistance. Biocontr. Sci. Technol. 2, 249-351

Martinez-Ochoa, N., Kloepper, J.W. and Rodríguez-Kábana, R. (1995). PGPR-mediated induced systemic resistance against root-knot nematode (*Meloidogyne incognita*) on cucumber. Phytopathology 85, 1154 (abstract).

Maurhofer, M., Keel, C., Haas, D. and Defago, G. (1995). Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHAO with enhanced antibiotic production. Plant Pathol. 44, 40-50.

... A., Hallmann, J. and Sikora, R.A. (2000). Evaluation of the biocontrol activity of endophytic bacteria from tomato against *Meloidogyne incognita*. Med. Fac. Landbouww. Univ. Gent 65(2b), 471-480.

Pleban, S., Ingel, F. and Chet, I. (1995). Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp. European J. Plant Pathol. 101, 665-672.

Reitz, M., Rudolph, K., Schröder, I., Hoffmann-Hergarten, S., Hallmann, J. and Sikora, R.A. (2000). Lipopolysaccharides of *Rhizobium etli* G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. Applied and Environ. Microbiol. 66, 3515-3518.

Sikora, R.A. (1988). Interrelationship between plant health-promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Med. Fac. Landbouww. Univ. Gent 53(2b), 867-878.

van Loon, L.C., Bakker, P.A.H.M. and Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36, 453-483.

van Peer, R. Nieman, G.J. and Schippers, B. (1991). Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81, 728-734.

Wei, G., Kloepper, J.W. & Tuzun, S. (1996). Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86, 221-224.

Zehnder, G.W., Changbin, Y., Murphy, J.F., Sikora, E.R., Kloepper, J.W., Schuster, D.J. & Tuzun, J.E. (1999). Microbe-induced resistance against pathogens and herbivores: evidence of effectiveness in agriculture. In: A. Agrawal, S. Tuzun & E. Bent.(Eds.) Induced Plant Defenses against Pathogens and Herbivores: Biochemistra, Ecology and Agriculture. APS Press, St. Paul, MN, pp.335-355.