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Understanding Fungus-Nematode Interactions to Control the Meloidogyne Javanica Species

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Abstract – This study was aimed at elucidating the parasitic capabilities of Trichoderma isolates on the RKN, M. javanica and their biocontrol activities against the nematode. Parasitism is probably an important mode of action and one of the initial steps of this process is attachment. The nematode's gm enabled fungal attachment and enhanced parasitic capabilities of the isolates (except T. harzianum), which could also utilize gm as a nutrient source. It has also been found to trigger proteolytic and chitinolytic enzyme production by the fungus (Sharon et al. unpubl.). This combination of enzymes is required to disrupt the eggshell, although chitinolytic capacity is probably the most important activity on the eggshells. Trichoderma asperellum GH-11 exhibited lower parasitic capabilities that might be related to insufficient proteolytic activity of this isolate, while T. atroviride presented the greatest efficiency for parasitism of J2s, probably because of its high proteolytic activities (Sharon et al. unpubl.). Production of proteinase Prb1 in this isolate has been studied and its involvement in fungal parasitism has been shown. A transgenic T. atroviride line (P2) containing multiple copies of the prb1 gene also exhibited improved biocontrol activity of M. javanica in soil experiments and in parasitism assays on agar in vitro.The gm also triggered an immobilization effect on J2s produced by T. atroviride and T. asperellum isolates 203 and 44. This effect might be the result of enzymes and metabolites, such as peptaibols, the activities of which may act synergistically.

Key words: Parasitism, Meloidogyne javanica, proteinase, biocontrol.

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INTRODUCTION

Parasitism is probably an important mode of action and one of the initial steps of this process is attachment. The nematode's gm enabled fungal attachment and enhanced parasitic capabilities of the isolates (except T. harzianum), which could also utilize gm as a nutrient source.

The gm has also been found to trigger proteolytic and chitinolytic enzyme production by the fungus (Sharon et al. unpubl.). This combination of enzymes is required to disrupt the eggshell although chitinolytic capacity is probably the most important activity on the eggshells (Morton et al. 2004). Trichoderma asperellum GH-11 exhibited lower parasitic capabilities that might be related to insufficient proteolytic activity of this isolate, while T. atroviride presented the greatest efficiency for parasitism of J2s, probably because of its high proteolytic activities (Sharon et al. unpubl.). Production of proteinase Prb1 in this isolate has been studied and its involvement in fungal parasitism has been shown (Flores et al. 1997). A transgenic T. atroviride line (P2) containing multiple copies of the prb1 gene also exhibited improved biocontrol activity of M. javanica in soil experiments and in parasitism assays on agar in vitro (Sharon et al. 2001). The gm also triggered an immobilization effect on J2s produced by T. atroviride and T. asperellum isolates 203 and 44. This effect might be the result of enzymes and metabolites, such as peptaibols, the activities of which may act synergistically.

REVIEW OF LITERATURE:

The technique appears to have been first used to transfer the Mi gene from wild Tomato into commercial cultivars by Smith (1944) in crossing *Solanum lycopersicum* var. Michigan State with *S. peruvianum* PI128.657.

Dr. Charles Rick and colleagues at UC Davis discovered that an isozyme, acid phosphatase, is coded by the gene Aps-1 which is located on chromosome 6 of Tomato close to, and tightly linked with, Mi (Rick and Fobes, 1974). The isozyme provides a tool for Tomato breeders to determine whether they have successfully transferred Mi into commercial varieties and has facilitated the development of processing varieties with root-knot nematode resistance.

The Mi gene has been cloned and sequenced in the laboratory of Dr. Valerie Williamson at UC Davis. Using *Agrobacterium* as a carrier, the resistance gene has been transferred to a susceptible Tomato

cultivar, which expresses the resistance. Plants grown from seeds of the transgenic plant are also resistant to *M. incognita*. However, after the second generation of plant offspring, the expression of resistance is progressively reduced in seed batches from some plants but not from others. In both cases, the gene is still present and is still coding for RNA (Goggin et al, 2004).

The resistance conferred by the Mi gene breaks down at soil temperatures >28C.

With repeated use of the single source of resistance in California Tomato production, aggressive strains of the nematode are being selected (Kaloshian et al. 1996).

In the early 1990s, farm advisors and entomologist Dr. Harry Lange noticed that Tomatoes with the Mi gene appeared to be also resistant to the Tomato aphid, *Macrosiphum euphorbiae*.

MATERIAL AND METHOD:

This study was aimed at elucidating the parasitic capabilities of Trichoderma isolates on the RKN, M. javanica and their biocontrol activities against the nematode. Parasitism is probably an important mode of action and one of the initial steps of this process is attachment. The nematode's gm enabled fungal attachment and enhanced parasitic capabilities of the isolates (except T. harzianum), which could also utilize gm as a nutrient source.

The gm has also been found to trigger proteolytic and chitinolytic enzyme production by the fungus (Sharon et al. unpubl.). This combination of enzymes is required to disrupt the eggshell (Tikhonov et al.2002; Khan et al. 2004), although chitinolytic capacity is probably the most important activity on the eggshells (Morton et al. 2004). Trichoderma asperellum GH-11 exhibited lower parasitic capabilities that might be related to insufficient proteolytic activity of this isolate, while T. atroviride presented the greatest efficiency for parasitism of J2s, probably because of its high proteolytic activities (Sharon et al. unpubl.). Production of proteinase Prb1 in this isolate has been studied and its involvement in fungal parasitism has been shown (Flores et al. 1997). A transgenic T. atroviride line (P2) containing multiple copies of the prb1 gene also exhibited improved biocontrol activity of M. javanica in soil experiments and in parasitism assays on agar in vitro (Sharon et al. 2001). The gm also triggered an immobilization effect on J2s produced by T. atroviride and T. asperellum isolates 203 and 44. This effect might be the result of enzymes and metabolites, such as peptaibols, the activities of which may act synergistically.

Parallel formation and synergism of hydrolytic

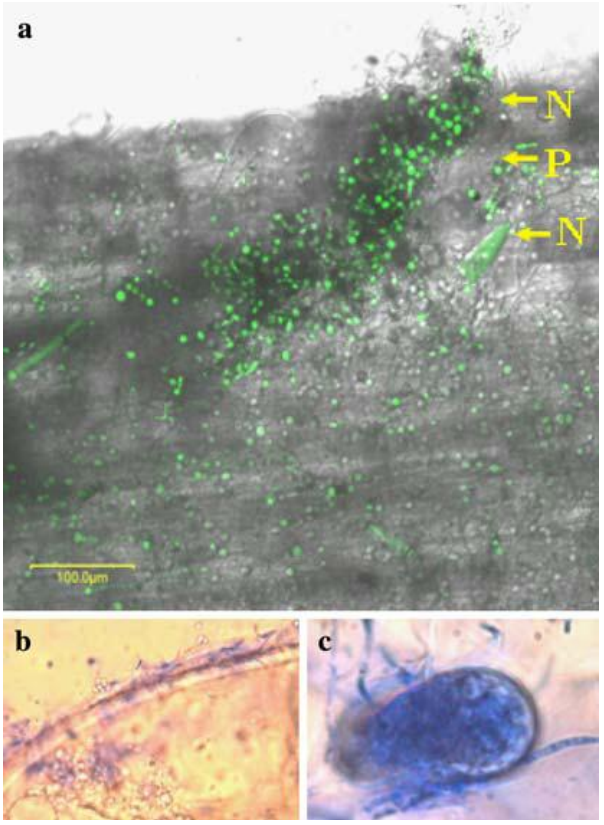

Fig. Interactions of Trichoderma asperellum-203 with Meloidogyne javanica developing stages in planta. (a) Colonization of Tomato roots by GFP-expressing construct and interaction with second-stage juveniles (J2s) during root penetration in sterile soil; nematode (N) penetration site (P) colonized by the fungus. A penetrating nematode showed green autofluorescence, probably indicating loss of viability. Bar = 100 mm. (b–c) Parasitism of T. asperellum-203 on mature nematode life stages dissected from Tomato roots grown in Trichoderma-treated soil: (b) Female infected by the fungus. (c) Egg within the egg mass colonized by the fungus. The fungus was stained with aniline blue enzymes and peptaibol antibiotic action against phytopathogenic fungi has been reported in Trichoderma (Schirmbo¨ck et al. 1994; Kubicek et al. 2001).

Conidial attachment and parasitic processes were microscopically monitored in vitro. Hyphal coiling, branching, enlargement of hyphal tips and appressoria- like structures were observed during parasitism on nematodes, resembling the mycoparasitic behaviour of Trichoderma (Chet et al. 1997). Nematode egg surfaces are infected by some fungal endoparasites, such as Pochonia chlamydosporia (Verticillium chlamydosporium) and Paecilomyces lilacinus, by producing appressoria laterally or at the tip of the hyphae growing across the eggs (Kerry and Hominick 2001). The biomimetic system successfully expressed the specific triggering of fungal attachment and parasitic growth patterns by the gm, similar to the parasitism on the nematodes. It was also similar to those induced by lectins derived from host fungi (Inbar and Chet 1994). Coiling on

nylon fibers has been induced by several commercial lectins, such as concanavalin-A (Con-A), wheat-germ agglutinin (WGA) and Ulex europaeus-I (gorse, UEA-I) (Rocha-Ramirez et al. 2002).

Hyphae of T. atroviride, which was the most effective parasite of the J2s, showed a higher tendency to coil around the J2s than those of T. asperellum-203. Similar results with respect to the coiling process have been obtained in fungal–fungal biomimetic interactions using nylon fibers, especially after induction with a G-protein activator (Omero et al. 1999). The signal-transduction pathways downstream of the recognition event have recently been intensively investigated, with a focus on the role of G-protein a-subunit genes (Zeilinger et al. 2005). Further investigations may determine whether similar pathways are involved in gm induction of fungal parasitic behaviour.

Fucose inhibited conidial attachment to J2s, conidial agglutination by gm suspension and their attachment to nylon fibers; attachment was also inhibited after periodate treatment of nematodes. It is suggested that carbohydrate lectin-like interactions might be involved in these processes; such interactions are sometimes Ca2+-dependent. This was the case for binding of red blood cells to nematodesurfaces, where the presence of C-type fucose- mannose- and glucose-binding proteins (carbohydrate- recognition domains, CRDs) on the J2s was suggested (Spiegel et al. 1995). The nematode surface coat also contains carbohydrate residues, including fucose and mannose (Spiegel and McClure 1995).

Information about the composition of the gm is scarce. Its amino-acid and amino-sugar contents have been analyzed and some glycoproteins have been characterized (Sharon and Spiegel 1993). A gm suspension was specifically labelled by several lectins, indicating the presence of carbohydrates such as fucose and N-acetyl-glucosamine (Sharon and Spiegel 1993). The conidial surface probably contains CRDs and carbohydrates: Elad et al. (1983) reported that attachment of T. asperellum-203 to Rhizoctonia solani is inhibited by galactose and fucose, and is Ca2+- and Mn2+-dependent. Following this work, Barak et al. (1986) isolated a fucose-binding agglutinin from the host fungus. We suggest that hatch from egg mass, gm, which contains carbohydrates such as fucose, binds to the J2s surface coat and this can alter their binding affinity to the fungal conidia that contain fucose-binding domains. As a result, gm-J2s are efficiently attached and parasitized by the fungus.

The results suggest that M. javanica gm plays a key role in the process of Trichoderma conidial attachment to the nematode and in the ensuing parasitism. The gm is usually considered a defensive envelope that protects the eggs against microorganisms and enables the egg mass to survive in the soil (Orion et al. 2001). Bacteria that were agglutinated by the gm could not reproduce in its presence, whereas others, which were not agglutinated, utilize the gm as a nutrition source and reproduce (Sharon et al. 1993). Thus, the ability of some Trichoderma species to be agglutinated by the gm and grow on it is unique, and partially accounts for their ability to attack RKNs; in contrast, the T. harzianum isolate was inhibited by the gm and was therefore not an effective parasite on the nematodes. The potential ability to parasitize nematode life stages in planta was demonstrated with T. as perellum- which interacted with penetrating J2 in a sterile soil system, and with females and egg masses on roots in soil, thereby interfering with the reproduction process. The potential parasitic capability of this isolate on the different nematode life stages may partially account for its high efficacy at reducing root galling and viable egg production in soil experiments.

The high affinity of this isolate as a root-surface colonizer (Yedidia et al. 1999) probably enhances these parasitic fungus-nematode interactions on the root surface. Kok et al. (2001) reported that the egg masses of Meloidogyne species from soils contained a bacterial community significantly greater than that of the rhizosphere. They suggested that the egg masses microflora may be an important factor in determining the success of nematode biocontrol agents. Interestingly, a strain of Trichoderma that strongly reacted against the biocontrol agent V. chlamydosporium wa found among M. fallax egg masses microflora. This work demonstrated the biocontrol activity of different Trichoderma isolates. Differences were observed among the isolates in their in vitro attachment and parasitic capabilities. Parasitism is one of the possible modes of action of Trichoderma against the nematodes; however, it is not always correlated with the biocontrol activity recorded among the different Trichoderma species and isolates, suggesting the involvement of other additional mechanisms.

Understanding fungus-nematode interactions and the mechanisms involved in the biocontrol process for various Trichoderma species and isolates might contribute to the development of improved biocontrol agents and their use with optimal implementation methods.

CONCLUSION:

We must recognise also that the effects of Bordeaux mixture in the 1880s, and of DDT in the 1940s, were *stunning.* Crop scientists were completely dazzled. As more and more of them began to abandon vertical resistance breeding, they chose crop protection chemicals because they were so dazzled. In comparison, there was nothing very dazzling about horizontal resistance. We should remember too that, during the whole of this century, crop scientists have

been faced with the world food problem. With the human population doubling every thirty years, crop scientists were compelled to double agricultural production every thirty years also. Much of that increase came from putting more land under the plough. Nevertheless, it was production, *per se,* that was given the first priority in crop science. The manner of that production was a secondary consideration. The corollary of this situation must also be recognised. There has been some truly remarkable progress in improving the yield, quality, and agronomic suitability of crops during the present century. The human population has increased dramatically, since the Mendelian school came into existence, yet we still produce enough food for everyone. The famines we have witnessed in recent years are due to local disasters, and to administrative incompetence, even political malice, rather than to a world shortage of food. The success of crop science in feeding the world has been impressive.

REFERENCES:

Ayala, A. (1962) j. agar. Univ. Peurto Rico. 46.

Azmi, M.I. (1986), Indian journal of Nematology.16.

Barak, R., Elad, Y., & Chet, I. (1986). The properties of L-fucose-binding agglutinin associated with the cell wall of Rhizoctonia solani. Archives of Microbiology, 144, 346–349.

Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia, 91, 553–556.

Chet, I., Inbar, J., & Hadar, Y. (1997). Fungal antagonists and mycoparasitism. In D. T. Wicklow & B. So¨derstro¨m

(Eds.), The mycota. Volume IV: Environmental and microbial relationships (pp. 165–184). Berlin, Heidelberg: Springer-Verlag.

Elad, Y., Barak, R., & Chet, I. (1983). Possible role of lectins in mycoparasitism. Journal of Bacteriology, 154, 1431– 1435.

Flores, A., Chet, I., & Herrera-Estrella, A. (1997). Improved biocontrol activity of Trichoderma harzianum by overexpression of the proteinase-encoding gene prb1. Current Genetics, 31, 30–37.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). Trichoderma spp.—opportunistic avirulent plant symbionts. Nature Microbiology Reviews, 2, 43–56.

Inbar, J., & Chet, I. (1992). Biomimics of fungal cell–cell recognition by use of lectin-coated nylon fibers. Journal of Bacteriology, 174, 1055–1059.

Inbar, J., & Chet, I. (1994). A newly isolated lectin from the plant pathogenic fungus Sclerotium rolfsii: purification, characterization, and its role in mycoparasitism. Microbiology, 140, 651–657.

Kerry, B. R., & Hominick, W. M. (2001). Biological control. In D. L. Lee (Ed.), Biology of nematodes (pp. 483–509).

London: Taylor and Francis.

Khan, A., Williams, K. L., & Nevalainen, H. K. M. (2004). nEffects of Pacecilimyces lilacinus protease and chitinase

on the eggshell structures and hatching of Meloidogyne javanica juveniles. Biological Control, 31, 346–352.

Kok, C. J., Papert, A., & Hok-A-Hin, C. H. (2001). Microflora of Meloidogyne egg masses: species composition, population density and effect on the biocontrol agent Eur J Plant Pathol (2007) 118:247–258 257

Verticillium chlamydosporium (Goddard). Nematology, 3, 729–734.

Kullnig, C., Krupica, T., Woo, S. L., Mach, R.L., Rey, M.,

Benitez, T., Lorito, M., & Kubicek, C. P. (2001). Confusion abounds over identities of Trichoderma biocontrol isolates. Mycological Research, 105, 770–772.

Kubicek, C. P., Mach, R. L., Peterbauer, C. K., & Lorito, M. (2001). Trichoderma: From genes to biocontrol. Journal of Plant Pathology, 83, 11–23.

Manzanilla-Lopez, R. H., Kenneth, E., & Bridge, J. (2004). Plant diseases caused by nematodes. In Z. X. Chen, S. Y.

Chen & D. W. Dickson (Eds.), Nematology—advances and perspectives. Volume II: Nematode management and utilization (pp. 637–716). Cambridge, MA: CABI Publishing. Morton, C. O., Hirsch, P. R., & Kerry, B. (2004). Infection of plant-parasitic nematodes by nematophagous fungi—areview of application of molecular biology to understand infection processes and to improve biological control. Nematology, 6, 161–170.

Omero, C., Inbar, J., Rocha Ramirez, V., Herrera Estrella, A., Chet, I., & Horwitz, B. A. (1999). G protein activators and cAMP promote mycoparasitic behaviour in Trichoderma harzianum. Mycological Research 103, 1637–1642.

Orion, D., Kritzman, G., Meyer, S. L. F., Erbe, E. F.,

Chitwood, D. J. (2001). A role of the gelatinous matrix in the resistance of root-knot nematode (Meloidogyne spp.) eggs to microorganisms. Journal of Nematology 33,

Rao, M. S., Reddy, P. P., & Nagesh, M. (1998). Evaluation of plant based formulations of Trichoderma harzianum for the management of Meloidogyne incognita on egg plant. Nematologia Mediterranea, 26, 59–62.

Reddy, P. P., Rao, M. S., & Nagesh, M. (1996). Managementof citrus nematode, Tylenchulus semipenetrans, by integration of Trichoderma harzianum with oil cakes. Nematologia Mediterranea., 24, 265–267.