0273-2289/94/4801—0037$03.00

Biological Control of Fungal Pathogens

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ABSTRACT

Biological control of soil-borne plant pathogens is a potential alternative to the use of chemical pesticides, which have already been proved to be harmful to the environment. Several strains of the fungus *Trichoderma* have been isolated and found to be effective biocontrol agents of various soil-borne plant pathogenic fungi under greenhouse and field conditions. Different application approaches have been used including integration of *Trichoderma* with reduced doses of chemical agents. Biochemical and molecular biology studies carried out to explore the mechanisms involved in biological control revealed that *Trichoderma* is a rather specific mycoparasite. Lectins were found to be involved in the recognition between *Trichoderma* and its host fungi, whereas chitinase is involved in the degradation of the host cell wall. Genetic engineering techniques were employed in order to increase the effectiveness, stability, and biocontrol capacity of *Trichoderma* spp. as well as other biocontrol agents, such as *Pseudomonass* spp. and *Rhizobium*.

Index Entries: Biological control; *Trichoderma* spp.; mycoparasitism.

INTRODUCTION

There is a growing concern in recent years, both in developed and developing countries, about the use of hazardous fungicides for controlling plant diseases. Chemical pesticides have already been proven to cause adverse environmental effects and result in health hazards to humans as

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well as other organisms including beneficial natural enemies. This concern, concomitantly with fascinating progress in biotechnological developments, has led researchers to develop safer and environmentally feasible control alternatives. Biological control, i.e., the use of biological processes to lower inoculum density of the pathogen in order to reduce the disease producing activities (*1*), thereby reducing crop loss, is a potential nonhazardous alternative. A direct approach in biological control of soil-borne plant pathogenic fungi involves the use of antagonistic microorganisms (e.g., fungi or bacteria) and applying them by different techniques. Research over the years went through screening for potential biocontrol agents and testing them under both greenhouse and field conditions, together with development of improved delivery systems. This research was accompanied by basic studies leading to better understanding of the mechanisms of control and applying this knowledge to improve existing biocontrol agents by means of genetic engineering and molecular biology. In the present article we summarize recent knowledge on biological control of plant pathogenic fungi with respect to these aspects of the research.

BIOLOGICAL CONTROL BY *TRICHODERMA* SPP: APPLICATION IN GREENHOUSE AND FIELD

Fungi from the genus *Trichoderma*, potential biocontrol agents, may be isolated from natural habitats of the target pathogens (i.e., infested soil or plants). This approach has, indeed, led to the isolation of several strains of *T. harzianum* antagonistic to and effective in controlling plant pathogenic fungi, such as *Rhizoctonia solani, Sclerotium rolfsii, Pythium aphanidermatum, Fusarium oxysporum,* and *F. culmorum,* both under greenhouse and natural field conditions. Several application techniques of *Trichoderma* have been developed including: broadcast application in infested fields; furrow application along the seedlings; loading the *Trichoderma* into the root zone before transplanting seedling to the field and seed coating with spore suspension of *Trichoderma* (*2,3*).

An isolate of *T. harzianum* (T-35) was isolated by Sivan and Chet (*4*) from the rhizosphere of cotton plants grown in field soil infested with *Fusarium*. Applying this isolate to the root zone of tomato or melon seedlings and transplanting them into commercial plots where a severe disease caused by either fusarium wilt of melon or fusarium crown rot of tomato had been recorded, reduced the incidence of the disease significantly and increased melon and tomato yields by 33 and 18%, respectively (*4,5*). Recently, *T. harzianum* was applied to cucumber and pepper seedlings as a peat-bran preparation (*6*) incorporated into the propagative mixture in a commercial production nursery. Significant increased growth effect of the *Trichoderma* on both crops were observed. Moreover, *Trichoderma*-treated plants were found to be more resistant to damping-off disease of

cucumber occurring during the first week after transplanting to a commercial greenhouse. In the first cycle, immediately after soil fumigation with methyl bromide (500 kg/ha), no damping-off was observed with either treatment, except in border beds where 4% of the nontreated plants died. In the second growing cycle, however, significant reductions in damping-off of 67 and 52% were obtained in middle and border beds, respectively, as compared to the nontreated controls (Inbar et. al., 1993, unpublished data).

INTEGRATED CONTROL

Soil fumigation with methyl bromide or Vapam is a commonly used technique to control soil-borne diseases. However, these methods, besides being time-consuming and uneconomical, pollute the atmosphere, and are environmentally harmful as the chemicals accumulate in the soil and water. Integration of biocontrol agents with reduced doses of chemical agents has a potential for controlling plant pathogens with minimal interference with the ecosystem and damage to the environment (*3*). Integrating *T. harzianum* and the fungicide captan signficantly reduced *Verticillium dahliae* colonization of the stem and increased potato yield, in fields naturally infested with this pathogen. The total yield of cv. Draga was increased by 15.7 and 46%, in first and second year, respectively, and 84% increase in marketable yield were obtained in the second year (*7*). Combination of *T. harzianum* with soil solarization or with a reduced dose of methyl bromide (300 kg/ha) under field conditions resulted in significant disease control of fusarium crown and root rot of tomato induced by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Disease control of 48% was obtained with a combination of *T. harzianum* and the reduced dose of the fumigant. Yield improvement of 105% over the control was recorded in plots where the antagonist had been applied in combination with soil solarization. Combining *T. harzianum* with methyl bromide or soil solarization resulted in 76 and 94% reduction in colonization of *Fusarium* spp. on the crown surface, respectively (*8*).

MECHANISMS INVOLVED IN THE BIOLOGICAL CONTROL OF FUNGAL PATHOGENS

Studying the mechanism involved in disease reduction revealed that *Trichoderma* acts as a mycoparasite (*9*) (Fig. 1). The *Trichoderma* recognizes and attaches to the pathogenic fungus and begins to excrete extracellular lytic enzymes, such as β-1,3-glucanase, chitinase, protease, and lipase. This recognition mechanism is the basis for the specificity of the antagonist. Lectins (i.e., carbohydrate binding proteins or glycoproteins), which

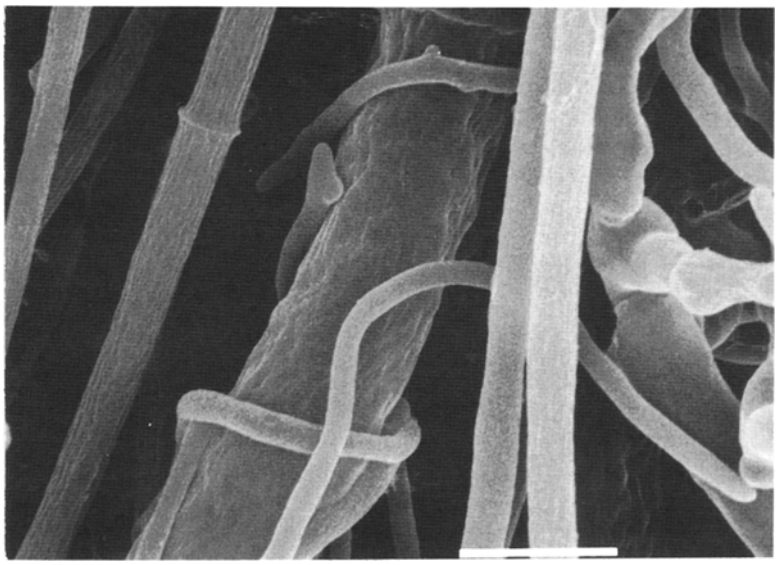

Fig. 1. Scanning electron micrograph (SEM) demonstrating mycoparasitic relationship between *Trichoderma* and the soil-borne plant pathogenic fungus, *Sclerotinia sclerotiorum*. *Trichoderma* hyphae coiled around *S. sclerotiorum* hyphae. Bar = 10 μm (Inbar, Menendez, and Chet, unpublished).

have been found to be produced by some soil-borne plant pathogenic fungi, such as *R. solani* and *S. rolfsii* (*10–12*) were thought to be involved in the recognition and specificity of the interaction between *Trichoderma* and its host fungi. Recently, using a biomimetic system, we were able to prove the role of lectins in mycoparasitism (*13*). When *T. harzianum* was allowed to grow on nylon fibers coated with concanavalin A or *S. rolfsii* lectin it coiled around the nylon fibers and produced hooks in a pattern similar to that observed with the real host hyphae (*13*) (Fig. 2).

Chitin is one of the main cell wall constituents of many plant pathogenic fungi. Evidence for the involvement of chitinase in the control of plant pathogenic fungi by both bacterial and fungal agents is becoming more and more convincing (*14–19*).

A chitinolytic isolate of *Aeromonas caviae* was isolated from roots of healthy bean plants grown in soil artificially infested in *S. rolfsii*. Under greenhouse conditions, the bacterium controlled *R. solani* and *F. oxysporum* f. sp. *vasinfectum* in cotton (78 and 57% disease reduction, respectively) and *S. rolfsii* in beans (60% disease reduction). There was no evidence of inhibition of the fungal pathogens by *A. caviae*. However, *A. caviae* partially lysed live mycelium of *R. solani*, *S. rolfsii*, and *F. oxysporum* f. sp. *vasinfectum* when their mycelium served as a sole carbon source in liquid medium. A high chitinolytic activity was found when colloidal chitin was used as sole carbon source. No β-1,3-glucanase was produced by the bacterium (*19*).

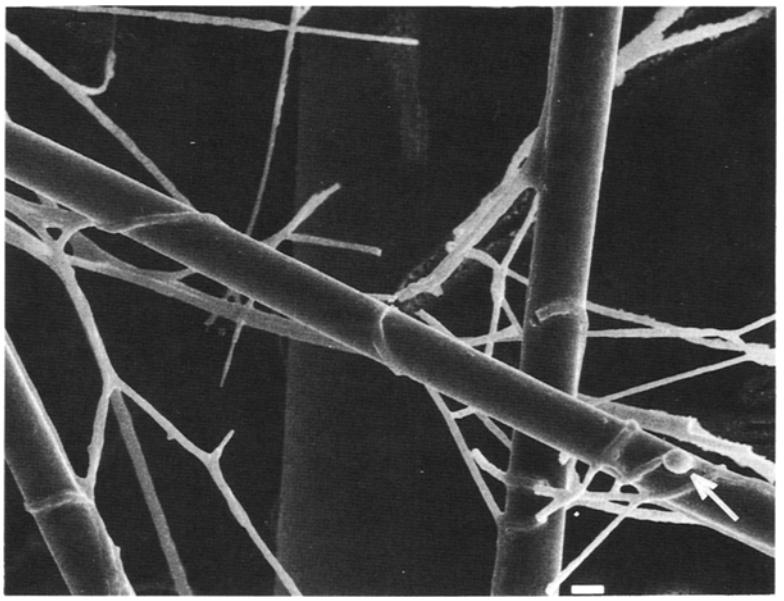

Fig. 2. SEM of *T. harzianum* mycelium grown on nylon fibers coated with the *S. rolfsii* lectin. *Trichoderma* coiled around the coated fibers in a similar pattern as with the real host. Appressorium like bodies (arrow) could be observed occasionally. Bar = 10 μm.

A method was developed for assessing the rhizosphere competence of this biocontrol agent by measuring chitinolytic activity along the intact roots or root segments using an image analyzer (*20*). When intact cotton roots were placed in Petri dishes containing solid medium with chitin as a sole carbon source, clearing zones appeared around the roots as a result of root-associated chitinolytic activity. Using this method, soil amendments with suspension of *A. caviae* was found to cause a significant increase in chitinolytic activity along the roots as compared with the untreated control. Dilution plate counts of root samples revealed that the biocontrol agent completely prevailed over the natural rhizosphere bacteria when applied by drench or by mixing with soil. A positive linear correlation was found between the number of chitinolytic bacteria present and the measured area of the clearing zone along the roots (*20*).

In an attempt to increase its effectiveness, *T. harzianum* protoplasts were cotransformed using two plasmids: pSL3chiAII, containing a bacterial chitinase gene from *Serratia marcescens* under the control of a constitutive viral promotor, and p35SR2, a marker for selection after transformation, encoding for acetamidase a marker for selection after transformation. Two transformants showed increased constitutive chitinase activity (specific activity, 11 and 5 times higher than the recipient) and excreted a protein of ca. 58 kDa, the expected size of *S. marcescens* chitinase, when grown on synthetic medium. Antagonistic activity of the transformants

was significantly higher than those of *T. harzianum* wt., as was evaluated by testing their ability to overgrown the plant pathogen *S. rolfsii* in dual cultures (*21*). Sitrit et al. (*22*) introduced the chitinase gene from *S. marcescens* into the plant symbiont *Rhizobium meliloti*, which colonizes the root nodules of alfalfa. Cell-free extracts of nodules colonized by the transcojugants expressed the chitinase gene, as was evident by Western blot, and exhibited antifungal activity, expressed by the lysis of *R. solani* hyphal tips treated with these extracts. Broglie et al. (*15*) in a pioneering work produced transgenic tobacco seedlings constitutively expressing a bean chitinase gene under the control of the cauliflower mosaic virus 35S promotor. These plants were fungal resistant with increased ability to survive in soil infested with the fungal pathogen *R. solani* and delayed development of disease symptoms (*15*).

The major advantage of such genetic manipulations is the ability to isolate genes from one strain and introduce them into other varieties of fungi or bacteria, thus enhancing the potency of biocontrol agents and making a single strain effective, stable, and consistent against more than one plant pathogenic fungus, without the negative effects of chemical pesticides (*23*).

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Chais Family Foundation.

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