BIOLOGICAL CONTROL OF WILT PATHOGENS WITH FUNGAL ANTAGONISTS

R. P. Larkin and D. R. Fravel
USDA/ARS
Beltsville, MD

Abstract

Numerous fungi and bacteria, including existing biocontrol strains with known activity against soilborne fungal pathogens, were tested for their efficacy in controlling Fusarium wilt diseases. Specific nonpathogenic strains of *Fusarium* spp., isolated from wilt-suppressive soils, were the most effective antagonists for the reduction of Fusarium wilt diseases of tomato, watermelon, and muskmelon, providing consistent and significant disease control (50-80% reduction of disease) in several repeated tests. Other organisms, including isolates of *Gliocladium virens* and *Trichoderma hamatum* also significantly reduced Fusarium wilt compared to disease controls (30-60% reduction). Ecological studies determined that induced systemic resistance is involved in the mechanism of action for selected nonpathogenic *Fusarium* antagonists, and that these antagonists are effective at low inoculum densities, at high pathogen densities, and under a variety of different environmental conditions. These isolates have potential for development as biological control agents for the control of Fusarium wilt diseases of numerous crops. The fungal antagonist *Talaromyces flavus* has potential for the biological control of Verticillium wilt diseases. Production of the extracellular enzyme glucose oxidase, and the subsequent release of hydrogen peroxide, is associated with biocontrol efficacy. Improved control by this antagonist may be possible through combinations with sublethal heat or fumigation treatments, which weaken the pathogen and make it more susceptible to the action of *T. flavus*.

Introduction

Fusarium and Verticillium wilt diseases, caused by pathogenic formae speciales of *Fusarium oxysporum* and *Verticillium dahliae*, respectively, can cause severe losses in a wide variety of crop plants. For most crops, current control measures of using available disease-resistant or tolerant varieties and/or chemical controls are not adequate or practical, and alternative control measures are needed. We at the USDA Biocontrol of Plant Diseases Laboratory are actively investigating the use of biological control as an alternative strategy for the management of these diseases. Two recent research programs in our lab have focussed on the use of fungal antagonists for the control of Fusarium and Verticillium wilt diseases of multiple crops.

Biocontrol of Fusarium Wilt

Objective and Approach

The objective of our current research program is to develop effective biological control of Fusarium wilt of tomato and other crops of economic importance. The approach to accomplish this was divided into three phases. The first phase was to isolate and identify effective antagonist strains, which consisted of examining existing biocontrol strains with known activity against soilborne fungal pathogens, as well as isolating and screening potential new antagonist strains from soil and plant roots. In the second phase, the conditions and requirements (or ecological characteristics) of the biocontrol interaction were investigated. These studies included determining the mechanisms of action, the antagonist-pathogen inoculum density relationships, effectiveness under varying environmental conditions (including different soiltypes, different moisture and temperature regimes, different pathogen isolates, etc.), and other interactions with the soil microbial community. The last phase involves the optimization of biocontrol processes, in which we apply the principles and insight gained through the previous research to obtain more effective and consistent biological control.

Methods

Various fungi and bacteria with known biocontrol activity against soilborne fungal pathogens, including isolates of *Fusarium* spp., *Trichoderma* spp., *Gliocladium virens*, *Pseudomonas* spp., *Burkholderia cepacia*, *Cladorrhinum foecundissimum, Laetisaria arvalis,* and *Stilbella aciculosa,* were obtained from the collection of the Biocontrol of Plant Diseases Laboratory and various other sources. Potential new antagonists were isolated from the roots and rhizosphere of tomato plants grown in field plots at the Beltsville Agricultural Research Center, MD. Screening trials were conducted initially on tomato with follow-up tests on tomato, watermelon, and muskmelon. Seeds were planted in soilless potting mix (Redi-Earth, Scott-Sierra, Inc., Marysville, OH) infested with the antagonist to be tested and grown in seedling plug trays. Fungal antagonists were added as chlamydospore and/or conidial suspensions at ~10^6 cfu/ml. Bacterial antagonists were added as cell suspensions of ~10^8/ml. Plants were grown 2 weeks then transplanted into a loamy sand field soil infested with the pathogen at 10^4 cfu/g soil. Each treatment consisted of 5 replicate pots with 4 plants/pot. Disease was monitored for 4-6 weeks and assayed as the percentage of wilted seedlings. *Fusarium oxysporum* was reisolated from the stems of wilted seedlings to confirm Fusarium wilt.

Results and Discussion

Individual isolates within all groups of the known biocontrol organisms, including isolates of *F. oxysporum, T. hamatum, G. virens, P. fluorescens,* and *B. cepacia,* significantly reduced Fusarium wilt of tomato in greenhouse tests (Table 1). Disease reductions ranged from 35-100% relative to the pathogen-infested controls. As a group, the most effective

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antagonists were the *Fusarium* isolates collected from wilt-suppressive soils, with 8 of 10 isolates tested reducing disease by 50-100%. Several other fungal antagonists, including *G. virens* strain Gl-3, Gl-15, and Gl-21, *T. hamatum* strain TRI-4, *L. arvalis* strain Zh-4, and *C. foecundissimum* strain Cf-1, also significantly reduced Fusarium wilt in these screening tests (Fig. 1). The most effective of these other fungal antagonists were *G. virens* strains Gl-21, which reduced wilt incidence by 68%, and *T. hamatum* strain TRI-4 (64% disease reduction). However, even these isolates were not quite as effective as Fusarium isolates CS-20 and CS-1, which reduced wilt by 92% and 77%, respectively, in a corresponding test (Fig.1).

Subsequent tests with the nonpathogenic *Fusarium* spp. determined they were equally effective in reducing Fusarium wilt disease (50-80% reduction) on multiple crops (tomato, watermelon, and muskmelon) (Table 2). Disease reduction by each isolate was consistent across all three host crops, indicating no host-specific effect on disease reduction. Isolates CS-1 and CS-20 were consistently the most effective isolates on all tests, resulting in > 80% disease reduction. The mechanism of action for selected isolates (CS-20, CS-1) was shown to involve induced systemic resistance (Larkin and Fravel, 1996a). These isolates were effective at low antagonist inoculum densities, at high pathogen densities, in a variety of soil types, and against multiple races, isolates, and pathogenic formae speciales (Larkin and Fravel, 1996a,b). Additional tests with these isolates also demonstrated efficacy under a wide range of temperatures and on several different cultivars with varying resistance to Fusarium wilt (Larkin and Fravel, 1997)

Conclusions

Our results indicate that these *Fusarium* isolates have potential for development as biological control agents. Research is continuing to improve the effectiveness and consistency of control by these antagonists through: 1) further evaluations of the mechanisms, conditions, and requirements for optimum biocontrol activity, 2) field tests to study the efficacy of biocontrol under natural field conditions, 3) integration of biocontrol with other control strategies, and 4) improved formulations and delivery systems. This research demonstrates that effective biological control of Fusarium wilt diseases is feasible and, with further research, has potential as a viable alternative to chemical control for several crops.

Biocontrol of Verticillium Wilt

The soil-inhabiting fungus *Talaromyces flavus* (anamorph *Penicillium dangeardii*) has been investigated as a biocontrol against *Verticillium dahliae*. *T. flavus* suppresses Verticillium wilt of eggplant in the field (Marois et al., 1982), while control on other crops, such as potato, has been less consistent. As with many biocontrol agents, multiple mechanisms are responsible for the observed control. *T. flavus* produces large quantities of extracellular glucose oxidase (Kim et al., 1988, 1990a). Hydrogen peroxide is one of the products of the reaction catalyzed by this enzyme. Microsclerotia of *V. dahliae* are killed at concentrations of hydrogen peroxide 100-fold lower than concentrations used to kill other common soil microbes (Kim et al., 1990b). A natural variant of *T. flavus* with reduced ability to produce glucose oxidase was also reduced in its ability to control the pathogen (Fravel and Roberts, 1991). An antibody specific for glucose oxidase from *T. flavus* was used to immunoprecipitate the enzyme from culture filtrates to demonstrate that glucose oxidase is the antifungal principal involved in death of microsclerotia of *V. dahliae* (Stosz et al., 1996). Mycoparasitism is also important in the control of *V. dahliae* by *T. flavus* (Fahima et al., 1992). Cell wall degrading enzymes such as chitinase, β-1,3-glucanase and cellulase contribute to the mycoparasitic ability of *T. flavus* (Madi, et al., 1997).

Because *T. flavus* provides only partial control of Verticillium wilt, integration of biocontrol with other control methods is being investigated. Ascospores of *T. flavus* are very resistant to heat and fumigation with metham sodium. Sublethal levels of heating (solarization) or metham sodium can be used to weaken the pathogen, making it more susceptible to the action of *T. flavus*. Sublethal heating (Tjamos and Fravel, 1995) or metham sodium (Fravel 1996) acted additively to reduce wilt of eggplant. Sublethal heating also reduced the ability of *V. dahliae* to melanize new microsclerotia, which may reduce the survival ability of the pathogen in soil. Since heating or fumigation is used at a reduced rate, these treatments are less ecologically damaging than current control methods. Other biocontrol fungi such as *Trichoderma harzianum* and *Fusarium oxysporum* have also demonstrated potential for control of Verticillium wilt.

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Table 1. Potential antagonist organisms tested for their ability to reduce Fusarium wilt of tomato in field soil transplant tests.

Organism group	Total isolates tested	Reduction of disease[z]		
		# isolates	Range of reduction	Average reduction
Known Biocontrol Isolates				
Fusarium spp.	10	8	50-100%	66.6%
Tricho. & *Gliocladium* spp	14	4	37-75%	57.3%
Pseudomonas spp.	6	2	30-63%	46.1%
Burkholderia spp.	10	5	19-57%	36.4%
Other	4	2	35-61%	49.2%
Field Isolates				
Bacteria	120	29	30-65%	43.2%
Fungi	60	14	40-66%	45.1%

[z] Values represent the number of isolates within each group that significantly ($P<0.05$) reduced disease incidence (relative to pathogen-infested control soil), the range of percent reduction of disease for effective isolates, and the average percent reduction of disease for all, effective isolates in that group.

Table 2. Isolates of nonpathogenic *Fusarium* spp. most effective in reducing Fusarium wilt of tomato, watermelon, and muskmelon in greenhouse tests.

Isolate	% Wilt	% Red.	Species	Source
Path. control	58.7 a[x]	0		
CS-2	29.3 b	50.3	*oxysporum*	Florida, SS [y]
CS-10	24.1 b	57.8	*oxysporum*	"
CS-6	23.1 bc	60.2	*solani*	"
Fo47	20.4 bc	64.7	*oxysporum*	France, SS[z]
CS-21	18.8 bc	67.4	*oxysporum*	Florida, SS[y]
CS-24	17.3 bc	69.6	*oxysporum*	"
CS-20	10.7 c	81.5	*oxysporum*	"
CS-1	10.6 c	81.6	*solani*	"

[x] Means represent average values over all three hosts (isolate × host interactions were not significant). Means followed by the same letter are not significantly different ($P<0.05$) according to Duncan's multiple range test.

[y] Suppressive soil (Larkin et al., 1993, 1996)

[z] Suppressive soil (Alabouvette et al., 1993)

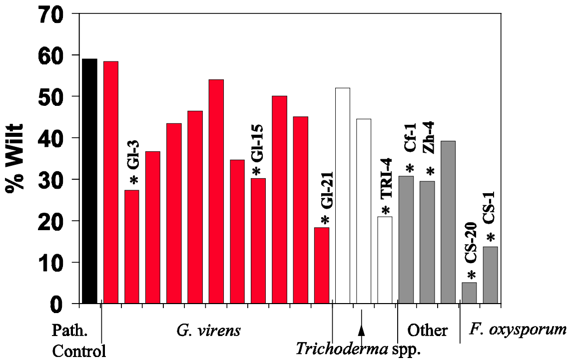

Figure 1. Development of Fusarium wilt in tomato seedlings as affected by biocontrol treatments of various antagonistic fungi. Bars topped by an asterisk denote significant reduction of disease relative to pathogen control.