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Antagonistic Properties of Gliocladium virens Against Wood Attacking Fungi

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SUMMARY

Gliocladium virens has shown good antagonism against decay fungi in agar medium and in wood blocks. Gliotoxin produced by G . virens is associated with biocontrol of some plant diseases, but its importance to biocontrol of wood-attacking fungi is unknown. We investigated the ability of gliotoxin-producing (GLT+) isolates of G . virens and gliotoxin-deffcient (GLT-) mutants of G . virens to inhibit growth of wood-attacking fungi in agar medium and to prevent decay in wood. The brown-rot fungi, Postia placenta and Neolentinus lepideus and the white-rot fungi, Trametes versicolor and Phlebia brevispora were completely inhibited by the GLT+ isolate and the GLT- mutants in agar medium. The GLT+ isolate also completely inhibited the growth of the brown-rot fungus, Gloeophyllum trabeum and the white-rot fungus, Irpex lacteus but the GLT- mutants caused lesser inhibition. The GLT+ isolate and GLT- mutants were ineffective in preventing growth of mold and stain fungi in dual agar culture. Pretreatment of wood blocks with a GLT+ isolate or GLT- mutant prevented decay by P . placenta. Likewise, the GLT+ isolate prevented decay by I . lacteus but the GLT- mutants did not. G . virens (GL-21) was grown on a sulfur containing medium l t pH 3.5 to enhance antibiotic production. However, inhibition of growth of decay fungi on agar medium containing culture filtrates was not enhanced. Decay was reduced in blocks treated with the culture filtrates but was not completely stopped. The filtrates were also ineffective in preventing growth of mold and stain fungi on wood.

INTRODUCTION

Gliocladium virens is one of the most widely studied genera of fungal biocontrol agents. It has proven effective as a suppressant of several soil-borne pathogens. G . virens has received little attention as a biocontrol agent for the control of wood attacking fungi. Highley and Ferge (1995), however, found that a commercially available biofungicide, G . virens (GL-21), was an effective pretreatment in the protection of wood against decay by all of the white-rot and brown-rot examined in laboratory tests. Although GL-21 produced metabolizes in an agar medium that inhibited growth of the decay fungi, wood treated with the metabolizes was not decay resistant nor was wood resistant to decay after death of GL-21.

Production of the antifungal antibiotics, gliotoxin and gliovirin, by G . virens has been associated with its efficacy as a biocontrol agent of most soil-borne diseases, but its importance to biocontrol of wood-attacking fungi is unknown. Howell and Stipanovic (1995) produced gliotoxin-deficient (GLT-)

mutants of G . virens to help ascertain the role of gliotoxin production in the G . virens / Rhizoctonia soloni biocontrol phenomenon. They found that biocontrol efficacy of the GLT- mutants to be equal to that of the parent strains, indicating that gliotoxin is not necessary for control of the disease.

The objective of this study was to compare the biocontrol efficacies of GLT- mutants of G . virens with that of parent strains in the prevention of fungal wood deterioration. This would help in understanding the role of fungitoxic metabolizes produced by G . virens in the control of wood deterioration.

METHODS

Fungi

The following Basidiomycete decay fungi were used:

Brown-rot- Postia (= Poria) placenta (Fr.) M. Lars. et. Lomb. [MAD-698], Neolentinus (= Lentinus) lepideus (Fr.:Fr:) Redhead and Ginns [MAD-534] and Gloeophyllum trabeum (Pers. :Fr.) Murr. [MAD-617]; white-rot- Phlebia brevisporia Nakas. in Nakasone et Eslyn [HBB-7030-sP], Irpex lacteus (Fr. :Fr.) Fr. [HBB-7328-sp.], Trametes (= Coriolus) versicolor L. ex Fr: Pilate [MAD-697], and Phanerochaete chrysosporium Burds. [ME-461].

The mold and stain fungi. used were:

Trichoderma sp., Aspirgillus niger van Tieghem, Penicillum sp., Ceratocystis pilifera (Fries) Moreau Ceratocystis minor (Hedgcock) Hunt, and Aureobasidium pullulans (deBary) Arnaud.

Gliocladium virens (Miller et al .) [GL-21] was donated by W.R. Grace and Co. in the form of dispersible granules.

G . virens strains G-11 and the UV-induced mutants, G-11-4 and G11-13, deficient for gliotoxin production, were provided by C.R. Howell, Southern Crops Research Laboratory, USDA/ARS, College Station, TX.

Tests for antagonistic effectiveness of G. virens strains in agar *medium*

Dual culture on malt-agar medium . A mycelial plug of the target fungus was placed on one edge of a Petri plate containing 2% malt extract in agar. Plates inoculated with mold or stain fungi were inoculated immediately on the opposite edge of the Petri plate with a G . virens strain. After 2 days, plates inoculated with the Basidiomycete fungi were inoculated with a G . virens on the opposite edge of the plate.

Filtrate inhibition . GL-21 was grown on a basal salts medium (Highley, 1973) with ammonium sulfate (0.2%) as the nitrogen source and glucose (l%) as the carbon source. The pH was adjusted to 3.5. Cultures were incubated for 7 days at 27°C and 70 percent relative humidity. Mycelial mats were removed

over a Buchner funnel and the filtrate sterilized through a 0.20 um sterile Millipore filter. Ten ml of the filtrate were placed in Petri dishes (90 mm) and 10 ml of the nutrient medium (2X) were added and mixed with the filtrate. Plates were inoculated with agar plugs of the decay fungi. Nutrient agar plates without the filtrate were inoculated with decay fungi to serve as controls. Cultures were incubated at 27°C, and colony diameter was periodically measured until the control fungi completely covered the plate.

Tests for antagonistic effectiveness of G. virens strains in wood

Treatment of wood blocks with G. virens strains

Southern pine or sweetgum test blocks (25mm X 25mm X 9mm, with the 9mm dimension in the grain direction) were treated with the G . virens strains and tested for decay resistance by the soil-block method. To determine if 1ive G . virens is necessary to render blocks resistant to decay, sterile filtrates (concentrated and unconcentrated) of GL-21, grown as above, were used to treat blocks. Green maple and southern pine blocks (3 X 28 X 42mm with the 3mm dimension in the grain direction) were also dip treated with sterile filtrates of GL-21 and exposed to mold and stain fungi.

Blocks vacuum impregnated or dip treated with culture filtrate. Gl-21 was grown in 500-ml flasks containing 100ml of the basal salts medium containing ammonium sulfate and 1% glucose. Cultures were incubated for 7 daya at 27°C and 70 percent relative humidity, blended for 10 seconds in a Waring blender, filtered through a Buchner funnel and the filtrate sterilized through 0.45um and 0.20um sterile Millipore filters. The total volume of filtrate obtained was 2000 ml. The entire solution was lyophilized and reduced to 200 ml. This was again filtered through a 0.45um sterile millipore and then further filtered through a 0.22um sterile filter. The solution was reduced to 1/10 the original volume and was taken as the high concentration. The l/l0 solution was added with equal volume of sterile water so as to obtain a concentration of 1/5 the original solution. This was taken as the low concentration but is certainly higher than the original filtrate solution. For decay tests, steam sterilized blocks were vacuum impregnated with the sterilized filtrate and dried down in absorbent lined pans for 3 hours prior to exposure to decay fungi in soil-block tests for six weeks. For mold and stain tests, blocks were steam sterilized and then dipped for one minute in the sterile filtrate preparations.

Blocks treated living G. virens strains . The G . virens strains, G-11, G-11-4 and G11-13 were cultured as above for 1 week. The cultures were blended for ten seconds in a Waring blender. Test blocks were vacuum impregnated with the blended culture and exposed to P . placenta and I . lacteus in soil-block tests for 10 weeks.

Wood-block tests

Blocks were evaluated for decay resistance using the standard American Society for Testing and Materials (1971) soil-block test. Bottles were incubated at

27°C and 70 percent relative humidity in the dark. Percentage weight loss, the measure of decay, was calculated from the weights of decayed blocks after equilibration at 70 percent relative humidity and 27°C. Original weights had been recorded after similar equilibrations. Five replications were used for each treatment.

Blocks treated with sterile culture filtrate were evaluated for resistance to stain and mold attck by placement in sterile 140mm diameter petri plates-lined with 3 layers of white paper towels maturated with water. Plates were inoculated with a spore suspension of the test fungi, placed in plastic bags and incubated for 8 weeks at 27°C and 70 percent relative humidity.

RESULTS

The gliotoxin-producing G . virens (GLT+) strain quickly overgrew the decay fungi and completely inhibited their growth in dual culture on a malt-agar medium [Table 1]. The gliotoxin-deficient [GLT-] mutants also completely inhibited the decay fungi P . placenta, N . lepideus , T . versicolor and P . brevispora in dual culture but caused lesser inhibition to G . trabeum and I . lacteus . Both the GLT+ and GLT- strains were ineffective in preventing growth of the mold and stain fungi in dual culture malt-agar medium.

Growth of the decay fungi on agar medium containing sterilized culture filtrate from GL-21 [GLT+] [prepared from a sulfur-containing medium, pH 3.5] was slowed but not completely inhibited [Table 2]. The stain fungus, C . pilifera was unable to grow on the culture filtrate containing medium but the culture filtrate had little effect on the growth of the other mold and stain fungi.

Pretreatment of wood blocks by vacuum impregnation with a blended culture of the GLT+ G . virens strain prevented decay the brown-rotter, P . placenta and the white-rotter, I . lacteus [Table 3]. Decay by P . placenta was also prevented by the GLT- strains of G . virens but the GLT- strains did not prevent decay by I . lacteus , although one GLT- strain slowed progression of decay.

Decay was reduced *in* blocks treated with a sterile filtrate from GL-21 cultured on a sulfur-containing medium, pH 3.5 to promote antibiotic production, but was not completely stopped [Table 4]. Concentration of filtrate further reduced the amount of decay in blocks but weight loss still occurred. Treatment of wood with the above filtrates was ineffective in preventing growth of stain and mold fungi.

DISCUSSION

The development of successful strategies for the use of biocontrol agents to protect wood from fungal attack will require a detailed knowledge of the mechanisms employed by the biocontrol agents to effect control. Gliocladium species produce a number of well-established antifungal agents, such as gliovirin and gliotoxin, often ascribed to the biological activity of

Gliocladium spp. We report in this paper the contribution of qliotoxin to the control of wood-attacking fungi by using gliotoxin-deficient mutants of G . virens to study biological efficacy against wood-attacking fungi.

Laboratory bioassays of GLT+ parent and GLT- mutant strains for efficacy to control wood decaying fungi showed that loss of gliotoxin had no effect on biocontrol performance of most of the wood decay fungi tested. Biological activity of the GLT- mutants was reduced against G . trabeum and I . lacteus . Thus, these results indicate that gliotoxin activity is not a primary mechanism in control of some wood decay fungi but may contribute to control of others.

Previously, we (Highley and Ferge, 1995) found that although treatment of wood blocks with living GL-21 prevented decay, treatment with sterilized culture filtrate (metabolites) was ineffective. Also, wood treated with GL-21 was not decay resistant after death of GL-21. Substrate and environment have a profound effect on secondary metabolize production by G . virens [Howell and Stipanovic, 1995). Sulfur-containing medium at low pH enhances antibiotic production by G . virens . Although treatment of blocks with sterilized culture filtrate from GL-21 *grown on* such a medium reduced decay, decay was not completely stopped, even when filtrates were concentrated. Thus, this is further evidence that the mechanism employed by G . virens to prevent wood decay involves mechanisms in addition to production of fungitoxic metabolizes. Competition may play an important role in the mechanism of decay prevention by G . virens similar to that suggested with the soil-borne disease R . soloni (Howell and Stipanovic, 1995).

G . virens continues to show promise as a biocontrol treatment to prevent wood decay. However, the results thus far indicate that neither living G . virens or its metabolizes are effective in preventing growth of mold and stain fungi that colonize green wood.

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Table 1. Interaction of wood-attacking fungi. with *Gliocladium virens* [GLT+] or gliotoxin-deficient [GLT-] mutants in agar medium

Fungus	Inhibition of growth (percent)		
	Gliocladium virens isolates		
	G-11 [GLT+]	G-11-4 [GLT-]	G-11-13 [GLT-]
	% Inhibition		
Brown-rot			
Postia placenta	100	100	100
Neolintinus lepideus	100	100	100
Gloeophyllum trabeum	100	72	62
White-rot			
Trametes versicolor	100	100	100
Phlebia brevispora	100	100	100
Irpex lacteus	100	62	65
Mold and stain			
Trichoderma sp.	0	0	0
Aspirgillus niger	0	0	0
Pencillium sp.	0	0	0
Certocystis pilifera	76	66	60
Certocystis minor	56	50	54
Aureobasidium pullulans	85	85	71

Table 2. Fungistatic ability of culture filtrate[a] from GL-21 in agar medium

Fungus	Inhibition of growth (%)
Brown-rot	
Postia placenta	80
Gloeophyllum trabeum	56
Neolentinus lepideus	87
White-rot	
Trametes versicolor	79
Phlebia brevispora	24
Irpex lacteus	43
Mold and stain	
Trichoderma sp.	16
Aspergillin niger	0
Pencillium sp.	0
Ceratocystis pilifera	100
Aureobasidium pullulens	29

[a]Filtrate from GL-21 grown on a basal salts medium with 0.20% ammonium sulfate as nitrogen source and pH adjusted to 3.5.

Table 3. Decay resistance of wood blocks treated with *Gliocladium virens* [GLT+] or gliotoxin-deficient (GLT-) mutants

Wood decay fungus	Weight loss[a] produced by wood-rotting Basidiomycete (percent)			
	Treatment method			
	Control	G-11 (GLT+)	G11-4 (GLT-)	G11-13 (GLT-)
	Southern pine			
Brown-rot *Postia placenta*	53.0±5.6	0	0	0
	Maple			
White-rot *Irpex lacteus*	61.8±6.3	0	32.5±24.4	63.5±7.5

[a]Weight loss with standard deviation (10 weeks exposure)

Table 4. Decay resistance of blocks treated with sterilized GL-21 filtrates

Wood decay fungus	Weight loss[a] produced by wood-rotting Basidiomycete (percent)		
	Control (untreated)	Filtrate (low conc)	Filtrate (high conc)
		Southern Pine	
Brown-rot			
Postia placenta	26.5±2.0	8.9±1.8	4.7±2.2
Gloeophyllum trabeum	26.7±2.4	14.9±2.9	14.1±2.4
		Sweetgum	
White-rot			
Phanerochaete chrysosporium	54.9±8.6	49.4±6.5	15.6±12.5
Trametes versicolor	40.3±5.l	47.9±4.5	18.1±5.1

[a]Weight loss with standard deviation (6 weeks exposure)