The Nature and Application of Biocontrol Microbes II: *Trichoderma* spp.

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Understanding the Mechanisms Employed by *Trichoderma virens* to Effect Biological Control of Cotton Diseases

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After 25 years of working with bacteria, actinomycetes, and fungi as biocontrol agents of cotton seedling and root diseases, I believe *Trichoderma virens* to be one of the most versatile and effective biocontrol agents we have studied. The species contains an arsenal of mechanisms and antimicrobial metabolites that give it a wide spectrum of activity against different pathogens (1,6,7, 22,29,30), and the capacity to control a number of diseases (4,6, 7,9,20,29,30).

To a biocontrol scientist, one of the salient features of *Trichoderma virens*, and one of the reasons it has been so frequently studied, is that it is an aggressive mycoparasite of other fungi (7,22,23), many of which are plant pathogens. It not only parasitizes the hyphae of many fungal species, but it also can penetrate and destroy some of the resting structures, thereby reducing their inoculum potential in soil (1,7,22). Mycoparasitism in *T. virens* has been associated with its capacity to synthesize extracellular chitinase (2). In addition to its mycoparasitic character, *T. virens* is also a vigorous saprophyte that can be cultured on any one of a number of substrates and can maintain itself in the absence of the host.

Concomitant with its mycoparasitic activities, *T. virens* also produces several potent epithiodiketopiperazine antibiotics. The antibiotic gliotoxin, (24) which is produced by "Q" strains of *T. virens*, has a wide spectrum of activity against bacteria, actinomycetes, and fungi. Gliotoxin has also been reported to act synergistically with chitinase in the antifungal activities of *T. virens* (3). The antibiotic gliovirin (16) produced by "P" strains of this fungus, although closely related to gliotoxin, has a much more restricted spectrum of activity, and it is not effective against bacteria, actinomycetes, or most fungi. It is, however, a potent inhibitor of oomycetes, such as *Pythium* and *Phytophthora* species. "Q" strains of *T. virens* do not produce gliovirin, and "P" strains do not produce gliotoxin (19). When either "P" or "Q" strains of *T. virens* are grown on substrates with high C/N ratios, they both produce the steroid-like compound viridiol. Viridiol has little or no antibiotic activity, but it is a potent phytotoxin when placed in contact with the roots of plants. With proper placement, i.e., mixed into surface soil, it can act as an effective herbicide for

weeds, without harm to the crop plant that is planted at greater depth (17). It has recently been discovered that strains of *T. virens* produce a mixture of peptaibols, linear peptide antibiotics, but their role in biological control is as yet unknown (25).

With antimicrobial mechanisms such as mycoparasitism and antibiotic production present, it would stand to reason that they play an important role in the biocontrol activities of this fungus. It was this hypothesis that guided most of our early research (1,7, 16,15,22–24,26). This, however, does not appear to be the case for cotton seedling diseases. When mutants of *T. virens* deficient for mycoparasitism, antibiotic production, or both were induced with UV light, they proved to be as biocontrol effective as the parent strains (8,18). In addition, *T. virens* strains grown on substrates that do not induce antibiotic production are just as effective as biocontrol agents of cotton seedling disease as those that are grown on substrates that do induce antibiotic production.

If the mechanisms of mycoparasitism and antibiosis are not vital to biological control by *T. virens*, what mechanisms are? By culturing fungi from the roots of treated cotton plants taken at intervals from field plantings throughout the growing season, we discovered that *T. virens* could be recovered from surface-sterilized roots at any time. The fungus colonized the tap and secondary roots in the upper reaches of the soil. However, late in the season it was found mostly on the tap and primary roots (28). Because the roots were rigorously surface-sterilized, we surmised that the fungus had penetrated the roots. This hypothesis was later supported by colleagues in Israel working with *T. harzianum*. They demonstrated microscopically that the fungus penetrated the epidermis and grew into the outer layers of the root cortex where it induced defense responses in cucumber (27).

We discovered that colonization of the cotton root by *T. virens* induced the synthesis of high levels of the terpenoid aldehydes hemigossypol (HG) and desoxyhemigossypol (dHG) that are intermediates in the pathway leading to synthesis of the dimer, gossypol. Bioassay of these compounds and gossypol against *Rhizoctonia solani* showed that both intermediates were strongly inhibitory to the pathogen, with dHG showing twice the toxicity of HG. Gossypol was only slightly inhibitory to *R. solani*. Bioassay of HG against *T. virens* and *T. koningii* showed that both fungi were resistant to the toxin (14). It was later learned that *T. virens* induced terpenoid synthesis in the cotton root by production of an 18-kDa protein that elicited both increased peroxidase activity and terpenoid concentrations in inoculated roots (5). Research has shown that there is a strong correlation between the

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ability of a strain of *T. virens* to induce terpenoid synthesis in cotton roots and its efficacy as a biocontrol agent of cotton seedling disease (14). Strains that do not induce terpenoid synthesis do not protect the root from subsequent infection by a pathogen. However, protection of the root by *T. virens* does not extend to the hypocotyl. No increase in peroxidase activity or terpenoid synthesis could be detected in the above-ground parts of *T. virens*-treated cotton plants (14).

Apparently, induction of phytoalexin synthesis in the roots of the cotton plant also protects it from the vascular wilt pathogens that enter through the root. Seed treatment of cotton with *T. virens*, and subsequent colonization of the developing root system, suppressed symptom development of Verticillium and Fusarium wilts of cotton when the plants were subsequently inoculated with the pathogens (4,30).

Another mechanism that *T. virens* employs in the biological control of the preemergence phase of cotton seedling disease that is incited by *Pythium ultimum*, *Pythium aphanidermatum*, or *Rhizopus oryzae* has recently been discovered. During the germination process, disease-susceptible cotton seeds release to the spermosphere compounds that stimulate resting structures of the pathogens in the surrounding soil to germinate and infect the seed. Seeds of resistant cultivars do not release these compounds. Seed treatment of susceptible cultivars with preparations of *T. virens* prevents infection of the seed by disrupting the process. The biocontrol agent coated onto the seed metabolizes the germination stimulatory compounds before they reach the pathogen propagules in the soil (10). Dead cells of *T. virens* are ineffective as biocontrol agents (11). The enzymes responsible for metabolism of the compounds that stimulate pathogen propagule germination are contained within the hyphal cells of *T. virens*. Exposure of the stimulatory compounds to cultures of *T. virens* destroys their stimulatory activity, while exposure to cell-free filtrates of *T. virens* cultures does not result in a loss of activity when the compounds are recovered and bioassayed (*unpublished data*).

The failure of cotton seed treatments with *T. virens* to induce phytoalexin synthesis in the above-ground parts of the plant leaves the hypocotyls unprotected and subject to postemergence damping-off by *Rhizoctonia solani*. Postemergence damping-off of cotton incited by *R. solani* can be controlled by application of the systemic fungicide Chloroneb 65W (1,4-dichloro-2,5-dimethoxybenzene) (21). However, this fungicide is not effective in the control of all preemergence pathogens. In soils where only pre- or postemergence pathogens occur, the biocontrol agent or the fungicide, respectively, can give effective control. In soils where both kinds of pathogens occur simultaneously, the most effective solution would appear to be a combination seed treatment containing both the fungicide and the biocontrol agent. However, Chloroneb is just as toxic in vitro to *T. virens* as it is to *R. solani*, leaving in question whether or not such a combination seed treatment is feasible. To test the efficacy of combination seed treatments in disease control, the seeds of a disease-susceptible cotton cultivar were first treated with one half the standard concentration of Chloroneb and air-dried. This was followed by coating the seed with latex sticker, and dusting the seed with a dry granular preparation of *T. virens*. These combination-treated seeds, along with untreated, Chloroneb-treated, or *T. virens*-treated controls, were planted in soil naturally infested with preemergence damping-off pathogens and amended with *R. solani*. After 7 days incubation at 25°C, seedlings from untreated, Chloroneb-treated, or *T. virens*-treated seeds did not emerge. Seedlings from combination-treated seeds emerged and remained healthy during subsequent development (12). Apparently, the systemic fungicide Chloroneb is absorbed by the seedling, protecting it from infection by *R. solani*. The latex polymer shields the *T. virens* in the biocontrol preparation from the effects of the fungicide.

When seeds of a highly susceptible cotton cultivar are treated with a "Q" strain preparation of *T. virens* and planted in soil

infested with preemergence damping-off pathogens, seedlings emerge normally and appear to be healthy. When seeds of the same cultivar are treated with preparations of "P" strains of *T. virens* and planted in infested soil, no emergence is observed. This difference between "P" and "Q" strains is not apparent with disease-resistant cultivars. The initial impression with susceptible cultivars is that the "P" strains are not capable of controlling the disease. However, when "P" strain-treated seeds are planted in noninfested soil, or in sterile vermiculite, they still do not emerge. This indicates that "P" strains of *T. virens* are pathogenic to susceptible cotton cultivars, while "Q" strains are not. Why is this so? A comparison of the enzymatic activities and viridiol (herbicide) production by "P" and "Q" strains indicates that there is little or no difference between the two, and seed treatment with the antibiotic gliovirin appears to have no effect on seed germination and seedling development. The answer to the puzzle appears to lie in the fact that "Q" strains induce high levels of phytoalexin production in cotton roots, while "P" strains do not. Taken together, these results indicate that both "P" and "Q" strains of *T. virens* are potential pathogens, but that cotton roots are able to contain further invasion by "Q" strains through production of induced phytoalexins. "P" strains do not induce sufficient phytoalexin production by cotton roots, and they are therefore much less restrained in their invasion and destruction of host plant tissue (15).

The ability of *T. virens* to act as a mycoparasite, synthesize antibiotic compounds, and to grow saprophytically at an impressive rate make it an effective competitor in the soil and root environment. On those pathogens whose resting structures are susceptible to infection by *T. virens*, the mycoparasite can be used to reduce pathogen inoculum potential in the soil. Other characteristics of *T. virens* make it an effective biocontrol agent of plant diseases when it is cultured, stored, and applied properly as a seed or soil treatment. The ability of "Q" strains to colonize and penetrate the epidermis and cortex of plant root systems, and to synthesize elicitor proteins, can lead to the induction of increased peroxidase activity and phytoalexin synthesis in the roots. These phenomena protect the developing plant root system from subsequent infection by root rotting and vascular wilt pathogens. The ability of *T. virens* strains to metabolize the pathogen propagule stimulatory compounds that are emitted by germinating cotton seedlings before they reach pathogen resting propagules is also an effective means of controlling preemergence seedling disease. It allows the seed to escape infection because the pathogen is not stimulated by the plant. If pathogen propagules are artificially stimulated to germinate, seed treatment with *T. virens* does not afford the plant any protection from early seedling disease. Lastly, the ability of *T. virens* to function as a biocontrol seed treatment in the presence of systemic fungicides coated on the seed (12,13) has greatly expanded the activity spectrum of the fungus as a biocontrol agent. Taken together, the characteristics exhibited by strains of *T. virens* make it one of the most effective and versatile biocontrol agents for seedling and root disease. The next steps in improving the biocontrol efficacy of *T. virens* strains are to expand their temperature/activity spectra and extend the storage period during which the biocontrol preparations remain effective.

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