Int. Microbiol (2001) 4: 1–4
DOI 10.1007/s101230100001

EDITORIAL

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# Understanding *Trichoderma*: between biotechnology and microbial ecology

Published online: 25 August 2001


Plant diseases, caused primarily by fungal and bacterial pathogens, causesevere losses of agricultural and horticultural crops every year. These losses can result in reduced food supplies, poorer-quality agricultural products, economic hardship for growers and processors, and, ultimately, higher prices. For many diseases, traditional chemical control methods are not always economical nor are they effective, and fumigation as well as other chemical control methods may have unwanted health, safety, and environmental risks.

Biological control involves the use of beneficial microorganisms, such as specialized fungi and bacteria, to attack and control plant pathogens and the diseases they cause. Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system. Biological control can be a major component in the development of more sustainable agriculture systems (Fig. 1). IPM is an approach to making pest and disease control decisions with increased information and involves the use of biological, physical, and chemical tactics to manage pest and pathogen populations in an economically efficient and ecologically sound way. However, within a given IPM strategy, the established view of biological control is that, even though it is safer than chemical control, it is less efficient and less reliable. To be realistic, we should not expect a very broad range of pest or disease control from biological agents, or that they control major pests or pest complexes in major crops in a wide range of environments. Biological control agents are, by their own nature, more limited than their chemical counterparts, and they need to be targeted carefully, starting with an appropriate characterization of the biocontrol agent and later selecting the antagonist for a given pathogen [7]. These types of considerations have encouraged microbiologists and plant pathologists to gain a better knowledge of biocontrol agents, to understand their mechanisms of control and to explore new biotechnological approaches.

Biocontrol agents can work several ways: (1) A biocontrol agent may grow faster or use its food source more efficiently than the pathogen, thereby crowding out the pathogen and taking over. The pathogens thus do not stand a chance! (2) A biocontrol agent may release a product that slows down or kills the pathogens in the vicinity of such a product; this process is called antibiosis. (3) A biocontrol agent may cause a plant to make a product that discourages or kills the pathogen; this process is called induced resistance. The plant actually fights back. (4) A biocontrol agent may feed directly on or in a pathogen; this process is called parasitism. In this way the pathogen is destroyed.

Some biocontrol agents use only one of these strategies but the most successful biocontrol agents use several of them. This is the case of the fungus *Trichoderma*. Most *Trichoderma* strains have no sexual stage but instead produce only asexual spores. For a few strains, the sexual stage is known; however, these do not include strains that have usually been considered for biocontrol purposes. The sexual stage, when found, is within the Ascomycetes in the genus *Hypocrea*. Traditional taxonomy was based upon differences in morphology, primarily of the asexual sporulation apparatus, but molecular approaches are now being used [2].

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**Fig. 1** Biocontrol trial with the *Trichoderma* formulation TUSAL

Consequently, the taxa recently have gone from consisting of nine to at least 33 species. As an example, the best biocontrol species, *T. harzianum* Rifai, has been separated into an array of species: *T. harzianum* s.str., *T. inhamatum*, *T. longibrachiatum*, *T. atroviride* and *T. asperellum* [4]. The improved knowledge of *Trichoderma* has facilitated the use of these microorganisms for biocontrol as whole cells, protein formulations and gene sources for transgenic plants.

## *Trichoderma* biocontrol strains

*Trichoderma* species have been investigated as biological control agents for over 70 years [8], but only relatively recently have strains become commercially available. This is largely a result of the change in public attitude towards the use of chemical pesticides, together with increased knowledge of their harmful side-effects. Biocontrol agents are widely regarded by the general public as "natural" and therefore non-threatening products. *Trichoderma* species act against target organisms in several ways [3]. Strains are actively parasitic on host fungi, through direct penetration of host hyphae and/or production of extracellular enzymes. In addition, species may produce antifungal antibiotics, and inhibition of pathogenicity-inducing hydrolytic enzymes has also been identified. Species may also be aggressive degraders of organic matter and act as competitors to fungal pathogens in their saprobic phases, especially when nutrients are a limiting factor. Some strains have been reported to promote the activities of saprobic bacteria and mycorrhizal fungi, while others act as plant-growth promoters, increasing plant size, foliar surface area and weight, and induce resistance towards plant pathogens. The dual roles of antagonistic activity against plant pathogens and promotion of soil fertility make *Trichoderma* strains appealing alternatives to hazardous fumigants and fungicides.

Many *Trichoderma* strains have been identified as having potential applications in biological control, and a partial list of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria*, *Botrytis*, *Chondrostereum*, *Colletotrichum*, *Dematophora*, *Diaporthe*, *Endothia*, *Fulvia*, *Fusarium*, *Fusicladium*, *Helminthosporium*, *Macrophomina*, *Monilia*, *Nectria*, *Phoma*, *Phytophthora*, *Plasmopara*, *Pseudoperonospora*, *Pythium*, *Rhizoctonia*, *Rhizopus*, *Sclerotinia*, *Sclerotium*, *Venturia*, *Verticillium*, and wood-rot fungi.

Once active strains have been identified using in vitro assays, a further selection must be done by studying other factors such as: (1) activity in vivo using experimentally induced diseases on plants, (2) tolerance of high temperatures (necessary to survive other IPM treatments), (3) suitability for formulation as foliar sprays and/or soil enhancements (e.g. high sporulation levels, rapid growth in bulk conditions), (4) specificity (strains should be inactive against beneficial organisms and plant crops), (5) long-term survival in field conditions, (6) interactions with other *Trichoderma* strains already present in the crop systems, (7) compatibility with agrochemicals used on the crop.

Commercial products currently on the open market or under registration include:

- Bio-Fungus (Belgium) against *Sclerotinia*, *Phytophthora*, *Rhizoctonia solani*, *Pythium* spp., *Fusarium*, *Verticillium*
- Trichodex (Israel) against *Botrytis* of vegetables and grapevines
- Binab-T (Sweden) for control of wound decay and wood rot
- Root Pro (Israel) against *R. solani*, *Pythium* spp., *Fusarium* spp., and *Sclerotium rolfsii*
- RootShield (also sold as Bio-Trek T-22G) (USA) against *Pythium* spp., *R. solani*, *Fusarium* spp.
- SoilGard (formerly GlioGard) (USA) for damping-off diseases caused by *Pythium* and *Rhizoctonia* spp.
- Supresivit (Denmark) against various fungi
- Trichoject, Trichopel, Trichodowels and Trichoseal (New Zealand) for control of *Armillaria*, *Botryosphaeria*, *Chondrosternum*, *Fusarium*, *Nectria*, *Phytophthora*, *Pythium*, *Rhizoctonia*
- TUSAL (Spain) for damping-off diseases caused by *Pythium*, *Phoma* and *Rhizoctonia* species, rhizomania disease of sugar beet and drop of lettuce
- Trichoderma 2000 (Israel) against *R. solani*, *S. rolfsii*, *Pythium* spp., *Fusarium* spp.
- Trieco (India) against *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp., root rot, seedling rot, collar rot, red rot, damping-off, *Fusarium* wilt

## *Trichoderma* protein formulations

*Trichoderma* strains have developed highly effective antagonistic mechanisms to survive and colonize the competitive environment of the rhizosphere, phyllosphere and spermosphere. One of its main mechanisms, mycoparasitism, relies on the recognition, binding and enzymatic disruption of the host-fungus cell wall. A major part of the *Trichoderma* antifungal system consists of a number of genes encoding for an astonishing variety of secreted lytic enzymes, including endochitinases, *N*-acetyl-$\beta$-glucosaminidases, chitin 1,4-$\beta$-chitobiosidases, proteases, endo- and exoglucan $\beta$-1,3-glucosidases, endoglucan $\beta$-1,6-glucosidases, lipases, xylanases, mannanases, pectinases, pectin lyases, amylases, phospholipases, RNases, and DNases [5]. Particularly useful for biocontrol applications are chitinolytic and glucanolytic enzymes because of their ability to efficiently degrade the cell wall of plant pathogenic fungi by hydrolyzing biopolymers not present in plant tissues. Each of these two classes of enzymes contains a number of proteins with different enzyme activity, and some of the enzymes have been purified and characterized and their genes cloned. Most of the enzymes tested as purified proteins have shown very strong antifungal activity, especially when assayed in combinations, against a variety of fungi. A substantial amount of work performed mainly during the past 7 years has indicated that cell-wall-degrading enzymes (CWDEs) from *Trichoderma* strains have great potential in agriculture as active components in new fungicidal formulations [1]. This is because purified CWDEs from different strains of *T. harzianum* are highly effective in inhibiting spore germination and mycelial growth in a broad range of pathogens such as *Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Venturia*, *Pythium*, *Phytophthora*, *Colletotrichum*, and especially *Botrytis*. In contrast to plant enzymes, chitinases and glucanases from *Trichoderma* can degrade not only the immature wall at hyphal apices but also the strong chitin-glucan complexes of mature cell walls, as well as survival structures such as sclerotia and chlamydospores, which reduces not only disease symptoms but also pathogen spread. In particular, enzymes absent from plants such as $\beta$-1,6-glucanases can degrade important fungal cell-wall structures such as $\beta$-1,6-glucans by linking chitin or $\beta$-1,3-glucans to cell-wall proteins. *Trichoderma* enzymes have diverse structural and kinetic properties, which increase the probability of avoiding inhibitory mechanisms.

The antifungal activity of *Trichoderma* CWDEs can be enhanced synergistically by combining enzymes with different lytic activities (such as exo- and endochitinases and/or glucanases). For instance, a combination of an endochitinase, an exochitinase and a $\beta$-1,3-glucanase purified from *T. harzianum* has an effective dose ($ED_{50}$) on *Botrytis* of about 1 ppm, which is comparable to the effective dose of most chemical fungicides. The inhibitory activity of chemical fungicides on *Botrytis* and other plant pathogens can be greatly enhanced by the addition of minute quantities (10–20 ppm) of *Trichoderma* CWDEs. For example, the fungicidal effect of azole compounds was enhanced up to 100-fold when used in conjunction with an endochitinase from *T. harzianum*, and a much greater improvement was obtained by adding small doses of two or more enzymes. Fungicides synergistic with the *Trichoderma* CWDEs include several compounds used for chemical control of plant diseases, such as azoles, benzimidazoles and pyrimidines. CWDEs from *Trichoderma* are synergistic with some plant pathogenesis-related proteins such as thaumatin-like proteins, which suggests that it is possible to use these enzymes as foliar sprays, enhancing natural plant defense mechanisms. Work on the antifungal activities of *Trichoderma* chitinolytic and glucanolytic enzymes has been performed primarily on *Botrytis*. Tests show that *Trichoderma* chitinases and glucanases have no effect on the plant even when relatively large quantities are injected into plant tissues.

CWDEs are not harmful to humans or animals, as indicated by EPA tests for registration of strains of *Trichoderma* for use as biocontrol agents in the United States, and they degrade into environmentally friendly residues. CWDEs can be effectively combined with whole-organism *Trichoderma* control, with considerable opportunities for synergism. CWDEs are particularly suited to post-harvest control. Low-temperature controlled storage conditions will favor these applications as the level of enzyme activities will be more easily predicted than in the greenhouse or the field. Purified CWDEs or mixtures of CWDEs with high antifungal activity obtained from *Trichoderma* culture filtrates can be included in commercial formulations since they are easily characterized, stable, resistant to drying, freezing, temperatures up to 60°C, and have broad pH and temperature optima. As a dry powder, they can be stored at room temperature for years without a major reduction in activity.

## ***Trichoderma*** **source of genes**

*Trichoderma* spp. have evolved numerous mechanisms for attacking other fungi and for enhancing plant and root growth. Several new general methods for biocontrol and for enhancement of plant growth have recently been demonstrated, and it is now clear that there must be hundreds of separate genes and gene products involved in the processes of mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogen's enzymes. Biocontrol microbes, almost by definition, contain many genes that encode products that permit biocontrol to occur. Several genes have been cloned from *Trichoderma* spp. that offer great promise as transgenes to produce crops resistant to plant diseases [6]. Transgenic expression of high levels of chitinolytic and glucanolytic *Trichoderma* enzymes do not affect plant morphology, development or yield, or infection by arbuscular mycorrhizal fungi. Most of these genes have been patented and are commercially available, but a number are in development to be used in agricultural biotechnology.

These genes, which are contained in *Trichoderma* spp. and in many other beneficial genomes, are the basis for future "natural" organic crop protection and production. To facilitate a healthier and cleaner agriculture is our challenge and goal, and only the best biotechnology tools will be the ones to reach farmers' hands.

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