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Review Article

Trichoderma–plant–pathogen interactions

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Abstract

Biological control involves the use of beneficial organisms, their genes, and/or products, such as metabolites, that reduce the negative effects of plant pathogens and promote positive responses by the plant. Disease suppression, as mediated by biocontrol agents, is the consequence of the interactions between the plant, pathogens, and the microbial community. Antagonists belonging to the genus *Trichoderma* are among the most commonly isolated soil fungi. Due to their ability to protect plants and contain pathogen populations under different soil conditions, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments. *Trichoderma* spp. also produce numerous biologically active compounds, including cell wall degrading enzymes, and secondary metabolites. Studies of the three-way relationship established with *Trichoderma,* the plant and the pathogen are aimed at unravelling the mechanisms involved in partner recognition and the cross-talk used to maintain the beneficial association between the fungal antagonist and the plant. Several strategies have been used to identify the molecular factors involved in this complex tripartite interaction including genomics, proteomics and, more recently, metabolomics, in order to enhance our understanding. This review presents recent advances and findings regarding the biocontrol-resulting events that take place during the *Trichoderma*–plant–pathogen interaction. We focus our attention on the biological aspects of this topic, highlighting the novel findings concerning the role of *Trichoderma* in disease suppression. A better understanding of these factors is expected to enhance not only the rapid identification of effective strains and their applications but also indicate the potentials for improvement of natural strains of *Trichoderma*.

Keywords: *Trichoderma*; Biocontrol; Elicitors; Three-way interaction; Induced systemic resistance; Secondary metabolites

1. Introduction

Traditional methods used to protect crops from diseases have been largely based on the use of chemical pesticides. Applications of fungicides and fumigants can have drastic effects on the environment and consumer, and are often applied in greater quantities than herbicides and insecticides in agricultural production. Chemical methods, are not economical in the long run because they pollute the atmosphere, damage the environment, leave harmful residues, and can lead to the development of resistant strains among the target organisms with repeated use (Naseby et al., 2000). A reduction or elimination of synthetic pesticide applications in agriculture is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on biocontrol agents (BCAs) for disease control alone, or to integrate with reduced doses of chemicals in the control of plant pathogens resulting in minimal impact of the chemicals on the environment (Chet and Inbar, 1994; Harman and Kubicek, 1998). To date, a number of BCAs have been registered and are available as commercial products, including strains belonging to bacterial genera such as *Agrobacterium*, *Pseudomonas*, *Streptomyces* and *Bacillus*, and fungal genera such as *Gliocladium, Trichoderma, Ampelomyces*, *Candida* and *Coniothyrium*.

Trichoderma spp. are among the most frequently isolated soil fungi and present in plant root ecosystems (Harman

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et al., 2004). These fungi are opportunistic, avirulent plant symbionts, and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease. So far, *Trichoderma* spp. are among the most studied fungal BCAs and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman, 2000; Harman et al., 2004; Lorito et al., 2004). Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages: (i) colonization of the rhizosphere by the BCA (''rhizosphere competence'') allowing rapid establishment within the stable microbial communities in the rhizosphere; (ii) control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms; (iii) improvement of the plant health and (iv) stimulation of root growth (Harman et al., 2004).

This review presents a compilation of the most recent advances in understanding the mechanisms involved in the interaction of *Trichoderma* spp. with phytopathogenic fungi and plants. We emphasize the biological and biochemical aspects of this topic, with particular attention paid to the molecular factors involved in the natural cross-talk occurring in soil and root environment. A better understanding of the principles regulating the interaction between fungal pathogens, host plants, and BCAs such as *Trichoderma* would enhance the practical application of these beneficial microorganisms for plant disease control.

1.1. *Trichoderma–pathogen interaction*

Trichoderma (teleomorph *Hypocrea*) is a genus of asexual fungi found in the soils of all climatic zones. *Trichoderma* is a secondary opportunistic invader, a fast growing fungus, a strong spore producer, a source of cell wall degrading enzymes (CWDEs: cellulases, chitinases, glucanases, etc.), and an important antibiotic producer. Numerous strains of this genus are 'rhizosphere competent' and are able to degrade hydrocarbons, chlorophenolic compounds, polysaccharides and the xenobiotic pesticides used in agriculture (Harman and Kubicek, 1998; Harman et al., 2004). The main biocontrol mechanisms that *Trichoderma* utilizes in direct confrontation with fungal pathogens are mycoparasitism (Papavizas, 1985; Harman and Kubicek, 1998; Howell, 2003) and antibiosis (Howell, 1998; Sivasithamparam and Ghisalberti, 1998).

1.1.1. *Mycoparasitism and lytic enzymes*

The complex process of mycoparasitism consists of several events, including recognition of the host, attack and subsequent penetration and killing. During this process *Trichoderma* secretes CWDEs that hydrolyze the cell wall of the host fungus, subsequently releasing oligomers from the pathogen cell wall (Kubicek et al., 2001; Howell 2003; Woo et al., 2006). It is believed that *Trichoderma* secretes hydrolytic enzymes at a constitutive level and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation (Harman et al., 2004; Lorito et al., 2006; Woo and Lorito, 2007—Fig. 1).

The molecular biology of the mycoparasitic interaction between pathogen and antagonist has been studied in detail. The factors activating the biocontrol gene cascade in *Tricoderma atroviride* strain P1 mutants containing the green fluorescent protein (*gfp*) or glucose oxidase (*gox*) gene reporter systems controlled by different inducible promoters (i.e. from the exochitinase *nag*1 gene or the endochitinase *ech*42 gene) have been evaluated. Interestingly, the expression of these genes involved in mycoparasitism was induced by the digestion products obtained

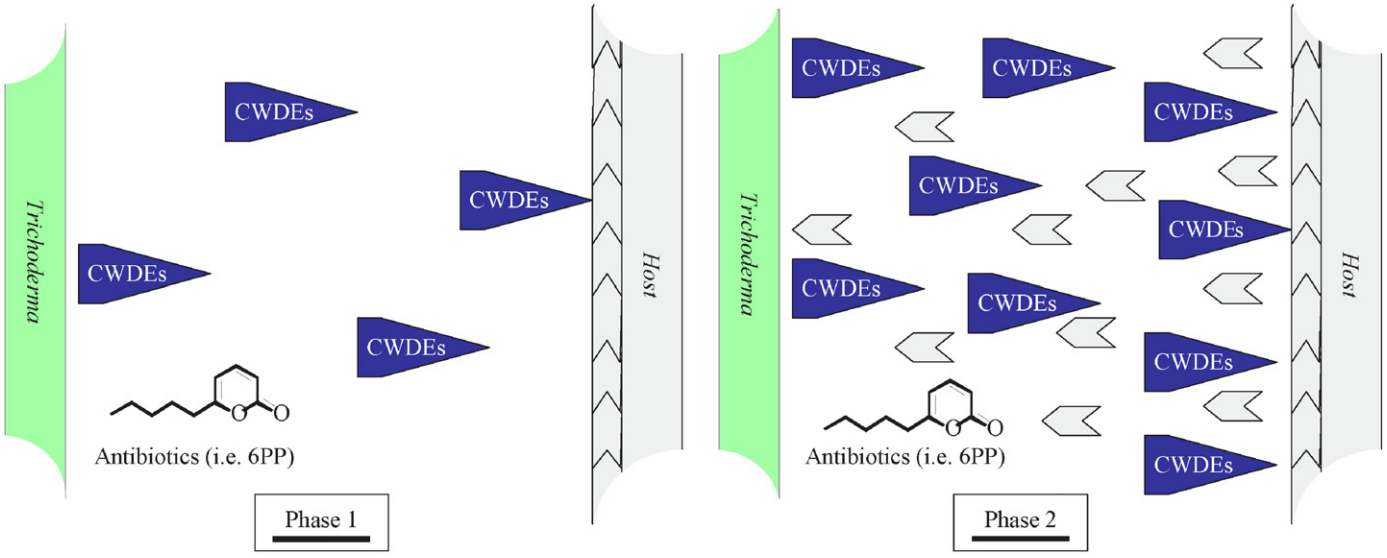


Fig. 1. The pre-contact events of the mycoparasitic interaction *Trichoderma*—host fungus. Phase 1: the mycoparasite produces high molecular weight compounds that reach the host. Phase 2: low molecular weight-degradation products that are released from the host cell walls reach the mycoparasite and activate the mycoparasitic gene expression cascade.

after treatments of fungal cell walls and colloidal chitin with purified CWDEs or fungal culture filtrates. LC/MS–MS analysis revealed that these novel mycoparasitism-related inducers have an oligosaccharide structure (Woo et al., 2004). Recently, the role of *Trichoderma* ABC transporters in both mycoparasitism and nutritional uptake by *Trichoderma* has been investigated (Ciliento et al., 2006). Unpublished but convincing data demonstrated that culture filtrates or mycelia of numerous plant pathogens induced the expression of specific *T. atroviride* ABC transporter genes, such as *tabc2*. This conclusion was confirmed by producing and analyzing knock-out mutants that showed a slower growth on different culture media or in presence of different fungal pathogens (*Botrytis cinerea*, *Rhizoctonia solani* and *Pythium ultimum*), as compared to the wild type strain (Ruocco and Lorito, unpublished).

The antifungal arsenal of *Trichoderma* spp. includes a great variety of lytic enzymes (Lorito, 1998; Lorito et al., 1994a, 1996a), most of which play a great role in biocontrol (Harman and Kubicek, 1998; Baek et al., 1999; Carsolio et al., 1999; Woo et al., 1999; Zeilinger et al., 1999; Kullnig et al., 2000; Kubicek et al., 2001). Many CWDEs from different *Trichoderma* strains have been purified and characterized (Lorito, 1998). Interestingly, when tested alone or in combinations, the purified proteins showed antifungal activity towards a broad spectrum of fungal pathogens (i.e. species of *Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Venturia* and *Colletotrichum,* as well as fungus-like organisms such as the Oomycetes *Pythium* and *Phytophthora* which lack chitin in their cell walls) (Tronsmo, 1991; Lorito et al., 1993, 1994a).

The direct application of anti-microbial compounds produced by fungal BCAs, instead of the whole "live" organisms, has numerous advantages in industry and agriculture, and may be more amenable to public opinion because of the inability of the agent to reproduce and spread. The selective production of active compounds may be performed by modifying the growth conditions, i.e. type and composition of culture medium, temperature of incubation and pH, etc. (Lorito and Scala, 1999; Woo and Lorito, 2007). The presence of different carbon sources, such as mono- or polysaccharides, colloidal chitin, or fungal tissues, has been shown to induce the secretion of CWDEs (Mach et al., 1999). Enhanced anti-fungal activity can be obtained by the combined application of *Trichoderma* enzymes with the fungus, different classes of synthetic fungicides, and in particular with compounds that affect the integrity of the cell membrane (Lorito et al., 1994b, 1996a). Moreover, purified mixes of CWDEs with different lytic activities showed improved antifungal effects against various plant pathogens, sometimes comparable to those obtained by using synthetic pesticides alone (Lorito et al., 1994b, 1996a; Baek et al., 1999; Carsolio et al., 1999).

1.1.2. Antibiosis and secondary metabolites

Trichoderma produces a plethora of secondary metabolites with biological activity (Ghisalberti and Sivasithamparam, 1991; Sivasithamparam and Ghisalberti, 1998). The term "secondary metabolite" includes a heterogeneous group of chemically different natural compounds possibly related to survival functions for the producing organism, such as competition against other micro- and macroorganisms, symbiosis, metal transport, differentiation, etc. (Demain and Fang, 2000). Included in this group are antibiotics, which are natural products able to inhibit microbial growth. Antibiotic production is often well correlated with biocontrol ability, and the application of purified antibiotics (Fig. 2) was found to show effects on the host pathogen similar to those obtained by using the corresponding living microbe. Ghisalberti et al. (1990) demonstrated that the biocontrol efficacy of *Trichoderma harzianum* isolates against *Gaeumannomyces graminis* var. *tritici* is related to the production of pyrone-like antibiotics.

The production of secondary metabolites by *Trichoderma* spp. is strain dependent and includes antifungal substances belonging to a variety of classes of chemical compounds. They were classified by Ghisalberti and Sivasithamparam (1991) into three categories: (i) volatile antibiotics, i.e. 6-pentyl-α-pyrone (6PP) and most of the isocyanide derivates; (ii) water-soluble compounds, i.e. heptelidic acid or koningic acid; (iii) peptaibols, which are linear oligopeptides of 12–22 amino acids rich in α-aminoisobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol (Pheol or Trpol) at the C-terminus (Le Doan et al., 1986; Rebuffat et al., 1989). The chemical structures of some of these compounds are reported in Fig. 3. Recently, we isolated and characterized the main secondary metabolites obtained from culture filtrates of two commercial *T. harzianum* strains (T22 and T39), and their production during the antagonistic interaction with the pathogen *R. solani* was also investigated (Vinale et al., 2006).

The chemical structures of *Trichoderma* antibiotics may suggest two different mechanisms of action. The production of low molecular weight, non-polar, volatile compounds (i.e. 6PP) results in a high concentration of antibiotics in the soil environment, that have a relatively long distance range of influence on the microbial community. On the contrary, a short distance effect may be due to the polar antibiotics and peptaibols acting in close proximity to the producing hyphae. Lorito et al. (1996b)

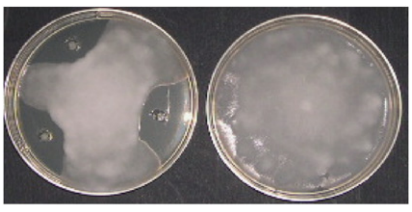
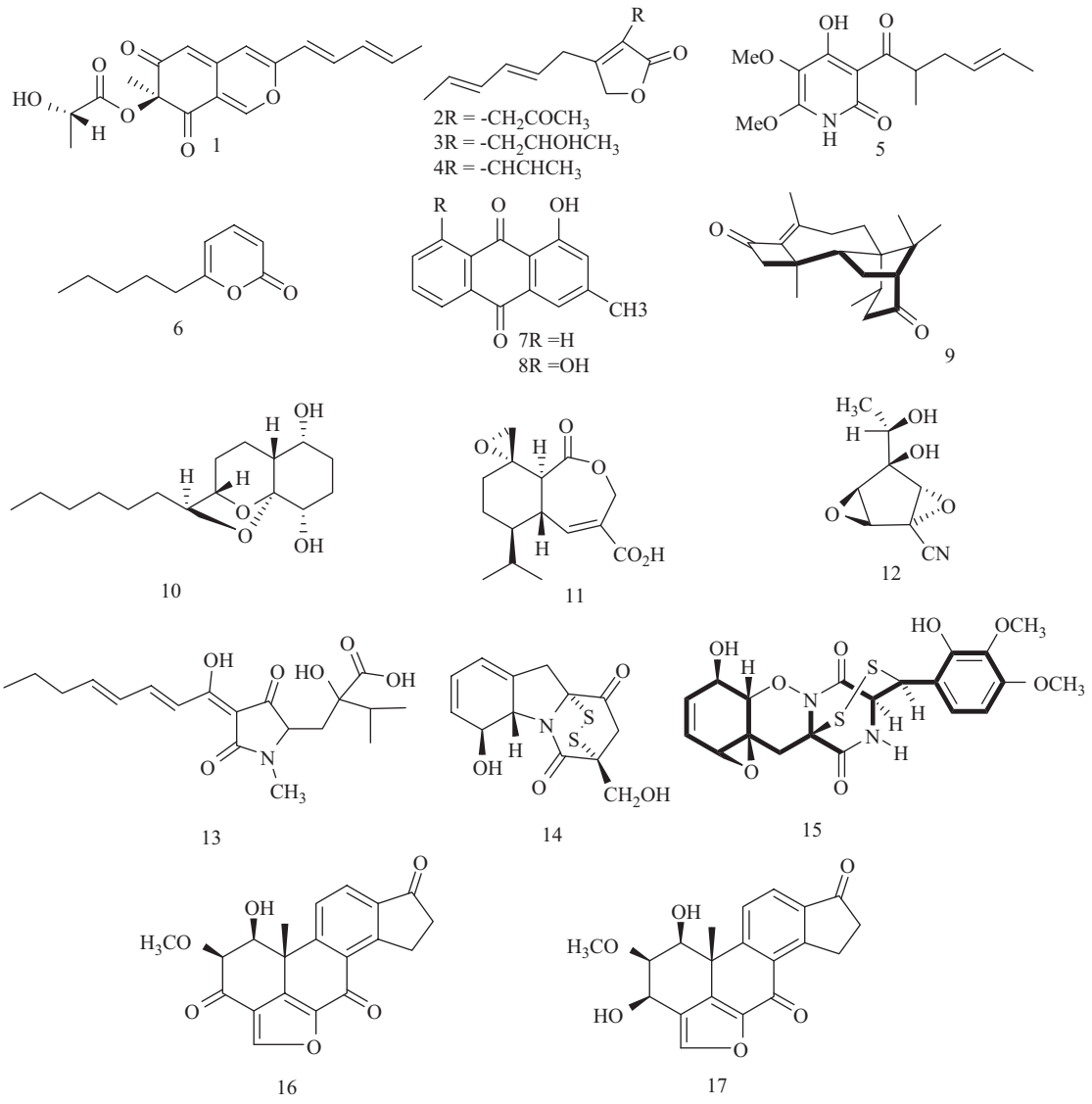


Fig. 2. Growth inhibition of *Pythium ultimum* by the antibiotic 6PP of *Trichoderma harzianum*. 1: medium containing 6PP; 2: medium not containing 6PP (control).



Ac-Aib-Ala-Ala-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Leu-Aib-Ile-Gln-Gln-Leuol

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Fig. 3. Chemical structures of secondary metabolites isolated from *Trichoderma* spp. 1: T22azaphilone; 2: T39butenolide; 3: harzianolide; 4: dehydro harzianolide; 5: harzianopyridone; 6: 6-pentyl-α-pyrone; 7: 1-hydroxy-3-methyl-anthraquinone; 8: 1,8-dihydroxy-3-methyl-anthraquinone; 9: harziandione; 10: koninginin A; 11: heptelidic acid; 12: trichoviridin; 13: harzianic acid; 14: gliotoxin; 15: glioviriN; 16: viridin; 17: viridiol; 18: trichorzianines.

demonstrated that peptaibols inhibited β-glucan synthase activity in the host fungus, while acting synergistically with *T. harzianum* β-glucanases. The inhibition of glucan synthase prevented the reconstruction of the pathogen cell wall, thus facilitating the disruptive action of β-glucanases. The synergism existing between enzymes and polar antibiotics is strictly related to their mechanism of action (Schirmböck et al., 1994; Lorito et al., 1996a, b; Fogliano et al., 2002). Although the role and the effects of peptaibols are clear, the mode of action of other *Trichoderma* secondary metabolites (i.e. pyrones), and their possible synergisms with other compounds have not yet been elucidated (Claydon et al., 1987; Serrano-Carreon et al., 1993; Howell, 1998).

According to the secondary metabolite produced, Howell et al. (1993) divided strains of *Trichoderma virens* into two groups: the "Q" strains able to produce the antibiotic gliotoxin and the "P" strains that produce a related compound, gliovirin, instead of gliotoxin (Howell and Stipanovic, 1983—Fig. 3, n. **14** and n. **15**). Gliotoxin has a broad spectrum of antibiotic activity, while gliovirin is a specific potent inhibitor of Oomycetes and its production was positively correlated with biocontrol efficacy of "P" group strains to control *Pythium* damping-off of cotton (Chet et al., 1997; Howell, 1998). On substrates with high C/N ratios, both "P" and "Q" strains of *T. virens* produce a phytotoxin similar to viridin, that is called viridiol (Fig. 3, **16** and **17**). The viridiol-producing

strains may be applied to surface soil as bio-herbicide for weeds, where they do not affect the crop plant that is planted in the treated soil (Howell, 2006). Other observations indicated that the biological control of pre-emergence damping-off by *T. virens* could be also related to its ability to degrade seed-emitted compounds that stimulate pathogen propagule germination (Howell, 2002). On the other hand, the induction of plant defence responses by some strains of *T. virens* plays a pivotal role in successful disease control of *R. solani* on cotton (Howell et al., 2000). In a recent study, Howell and Puckhaber (2005) indicated that "P" strains unable to induce the production of phytoalexins in cotton were ineffective as BCAs and pathogenic to susceptible cultivars. Conversely, "Q" strains inducing high levels of phytoalexin synthesis showed improved biocontrol efficacy and were not pathogenic to cotton roots. Phytoalexin synthesis in cotton is elicited by a protein produced by *T. virens* (Hanson and Howell, 2004), but the exact biochemical processes involved are not yet understood.

1.1.3. Competition with pathogens and soil microbial community

Competition for carbon, nitrogen and other growth factors, together with competition for space or specific infection sites, may be also used by the BCA to control plant pathogens. *T. harzianum* is able to control *B. cinerea* on grapes by colonizing blossom tissue and excluding the pathogen from its infection site (Gullino, 1992). Sivan and Chet (1989) demonstrated that competition for nutrients is the major mechanism used by *T. harzianum* to control *F. oxysporum* f. sp. *melonis*. Moreover, *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, thus making it more efficient and competitive than many other soil microbes (Benítez et al., 2004).

The biotic components of the soil environment have relevant effects on the biocontrol activity of *Trichoderma* against plant pathogens. Bae and Knudsen (2005), by using a *Gfp*-tagged mutant, showed that higher levels of microbial soil biomass induced a shift from hyphal growth to sporulation in *T. harzianum*, thus reducing its biocontrol efficacy. This effect may be associated with a phenomenon known as "soil fungistasis", which is largely dependent on the soil microbial community composition (de Boer et al., 2003). In particular, the production of antibiotic compounds and the presence of bacteria belonging to the genus *Pseudomonas* seem to be essential for the development of this phenomenon. In this context a detailed study of the metabolites produced by microorganisms present in the soil environment should be performed in order to avoid the suppression of BCAs.

1.2. Trichoderma–plant interaction

In addition to the beneficial effects that occur in direct interactions with plant disease agents, some *Trichoderma* species are also able to colonize root surfaces and cause substantial changes in plant metabolism (Harman et al., 2004). It is well documented that some strains promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman et al., 2004).

1.2.1. Plant root colonization

The physical interaction between *Trichoderma* and the plant was observed by electron microscopy to be limited to the first few cell layers of plant epidermis and root outer cortex (Yedidia et al., 1999). The hyphae of the BCA penetrate the root cortex but the colonization by *Trichoderma* is stopped, probably by the deposition of callose barriers by the surrounding plant tissues (Yedidia et al., 1999). It appears that this interaction evolves into a symbiotic rather than a parasitic relationship between the fungus and the plant, whereby the fungus occupies a nutritional niche and the plant is protected from disease. A very active, direct molecular cross-talk occurs between the fungus and the plant. Elicitors from *Trichoderma* activate the expression of genes involved in the plant defence response system, and promote the growth of the plant, root system and nutrient availability. This effect in turn augments the zone for colonization and the nutrients available for the biocontrol fungus, subsequently increasing the overall antagonism to plant pathogens (Yedidia et al., 2003; Hanson and Howell, 2004; Harman et al., 2004).

1.2.2. Plant growth promotion

Many BCAs, such as fungi, bacteria and viruses, are not only able to control the pathogens that cause plant disease, but are also able to promote plant growth and development. In greenhouse and field trials, the ability of *T. harzianum* T22 and *T. atroviride* P1 to improve the growth of lettuce, tomato and pepper plants under field conditions was investigated (Vinale et al., 2004) (Fig. 4). Crop productivity was increased up to 300%, as

Fig. 4. Plant growth promotion effects of *Trichoderma* spp. strains on: pepper (top), lettuce (lower left), and tomato (lower right) plants grown in the greenhouse.

determined by comparing the treated plots with the untreated controls and measuring fresh/dry root and above ground biomass weights, height of plants, number of leaves and fruits. This study also demonstrated the compatibility of *T. harzianum* T22 and *T. atroviride* P1 with pesticides conventionally used in organic farming by monitoring the effect on mycelia growth in both liquid and solid media. Results indicated a high level of tolerance by *Trichoderma* strains to concentrations of copper oxychloride varying from 0.1 up to 5 mM (Vinale et al., 2004; Fig. 5). These positive effects of *Trichoderma* may be obtained with different plant species, thus the genetic base of such interactions seems not to be predominant. Conversely, at least in maize the plant growth promotion effect is genotype specific and some inbreds respond negatively to different strains (Harman, 2006).

A yield increase was also observed when plant seeds were exposed to *Trichoderma* conidia that were separated from them by cellophane, suggesting that *Trichoderma* metabolites can influence the plant growth (Benítez et al., 2004). On the other hand, only a few reports deal with the ability of antagonistic fungal strains to produce compounds acting as growth promoting factors. Cutler et al. (1986, 1989) reported the isolation, identification and biological activity of secondary metabolites produced by *Tricoderma koningii* (koninginin A; Fig. 3, n. **10**) and *T. harzianum* (6-pentyl-α-pyrone; Fig. 3, n. **6**), that acted as plant growth regulators. Both metabolites significantly inhibited the growth of etiolated wheat coleoptiles at a relatively high concentration (10^{-3} M), but no effect was registered at lower doses (range from 10^{-4} to 10^{-3} M). It is hypothesized that such *Trichoderma* secondary metabolites may act as auxin-like compounds, which typically have an optimum activity between at 10^{-5} and 10^{-6} M while having an inhibitory effect at higher concentrations (Thimann, 1937; Cleland, 1972; Brenner, 1981), and/or are involved in the production of auxin inducers. The dose–effect response of such compounds on plant growth and development requires further investigation. *Trichoderma* spp. also produce organic acids, such as gluconic, citric or fumaric acids, that decrease soil pH and permit the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium, useful for plant metabolism (Benítez et al., 2004; Harman et al., 2004).

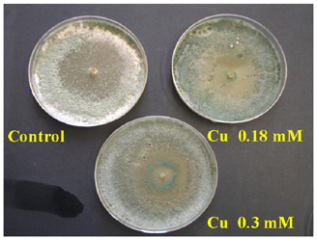

Fig. 5. Tolerance of *T. harzianum* T22 to copper oxychloride in potato dextrose agar medium at different concentrations.

1.2.3. Induction of plant defence responses

The induction of plant defence responses mediated by the antagonistic fungus has been well documented (De Meyer et al., 1998; Yedidia et al., 1999; Hanson and Howell, 2004; Harman et al., 2004). Various plants, both mono- and dicotyledonous species, showed increased resistance to pathogen attack when pre-treated with *Trichoderma* (Harman et al., 2004). Plant colonization by *Trichoderma* spp. reduced disease caused by one or more different pathogens, at the site of inoculation (induced localized acquired resistance, LAR), as well as when the biocontrol fungus was inoculated at different times or sites than that of the pathogen (induced systemic resistance or ISR).

The induction of plant resistance by colonization with some *Trichoderma* species is similar to that elicited by rhizobacteria, which enhance the defence system but do not involve the production of pathogenesis-related proteins (PR proteins) (Van Loon et al., 1998; Stacey and Keen, 1999; Harman et al., 2004). In a recent work Alfano and co-workers (2007) investigated at a molecular level the plant genes involved in *Trichoderma hamatum* 382 resistance induction by using a high-density oligonucleotide microarray approach. Interestingly, *Trichoderma*-induced genes were associated with biotic or abiotic stresses, as well as RNA, DNA, and protein metabolism. In particular, genes that codify for extensin and extensin-like proteins were found to be induced by the BCA, but not those codifying for proteins belonging to the PR-5 family (thaumatin-like proteins), which are considered the main molecular markers of SAR.

During the interaction of *Trichoderma* with the plant, different classes of metabolites may act as elicitors or resistance inducers (Harman et al., 2004; Woo et al., 2006; Woo and Lorito, 2007). These molecules include: (i) proteins with enzymatic activity, such as xylanase (Lotan and Fluhr, 1990); (ii) avirulence-like gene products able to induce defence reactions in plants (Woo et al., 2004); (iii) low-molecular-weight compounds released from fungal or plant cell walls by the activity of *Trichoderma* enzymes (Harman et al., 2004; Woo et al., 2006; Woo and Lorito, 2007). Some of the low-molecular-weight degradation products released from fungal cell walls were purified and characterized, and found to consist of short oligosaccharides comprised of two types of monomers, with and without an amino acid residue (Woo et al., 2006; Woo and Lorito, 2007). These compounds elicited a reaction in the plant when applied to leaves or when injected into root or leaf tissues. Further, they also stimulated the biocontrol ability of *Trichoderma* by activating the mycoparasitic gene expression cascade. Recently, Djonović et al. (2006) identified a small protein (Sm1) elicitor secreted by *T. virens*, and demonstrated its involvement in the activation of plant defence mechanisms and the induction of systemic resistance. In addition to their innate antimicrobial effect, their action may also stimulate the biological activity of resident antagonistic microbial

populations or introduced *Trichoderma* strains, and promote an ISR effect in the plant. Other secondary metabolites, like peptaibols, may act as elicitors of plant defence mechanisms against pathogens. In fact, application of peptaibols activated a defence response in tobacco plants (Benítez et al., 2004; Viterbo et al., 2006, personal communication). A peptaibol synthetase from *T. virens* was purified (Wiest et al., 2002), and the achieved cloning of the corresponding gene will facilitate an understanding of the role of this class of compounds in plant defence response.

1.2.4. Influence of soil environment on Trichoderma–plant interaction

The activities of BCAs are also affected by the presence of organic nutrients in soil (Hoitink and Boehm, 1999). Organic matter composition and the associated biotic and abiotic environment can affect the activities of *Trichoderma*, especially in relation to the conduciveness/receptivity of the soil to the strain (Simon and Sivasithamparam, 1989; Wakelin et al., 1999). So far, composts represent an optimal substrate for BCAs, thus encouraging their establishment into the soil environment (Hoitink and Boehm, 1999; Leandro et al., 2007). The mechanisms of action used by *Trichoderma* (competition, antibiosis, parasitism and systemic-induced resistance) are influenced by concentration and availability of nutrients (carbohydrates in lignocellulosic substances, chitin, lipids, etc.) within the soil organic matter (Hoitink et al., 2006). Krause et al. (2001) demonstrated that *T. hamatum* inoculation of potting mix with a high microbial capacity, which supported high populations of BCAs, significantly reduced the severity of *Rhizoctonia* damping-off of radish or *Rhizoctonia* crown and root rot of poinsettia. Moreover, *T. hamatum* inoculated into the compost amended potting mix induced systemic acquired resistance on cucumber, reducing the severity of *Phytophthora* leaf blight (Khan et al., 2004). This induction was more effective on plants grown in compost-amended media when compared to lower microbe carrying capacity sphagnum peat media (Hoitink et al., 2006). A better understanding of the activities of *Trichoderma* strains in plant growth media high in organic matter could also help to select strains suitable for multiple acre field conditions associated with stubble retention practices and/or organic farming which are becoming increasingly popular world-wide.

1.3. The three-way interaction: Trichoderma–plant–pathogen

The three-way interactions involving *Trichoderma*, plant and fungal pathogen have received less attention in comparison to the "simple" two-partner systems (i.e. plant–pathogen, plant–antagonist or pathogen–antagonist). There are obvious difficulties in studying such a complex system even if it is reproduced *in vitro*, although it better simulates the natural interactions occurring in soil agro-ecosystems. Recent studies have investigated some of the morphological or molecular aspects involved in plant–pathogen–antagonist interactions by using novel methods such as proteomics (Marra et al., 2006) and gene reporter systems (Lu et al., 2004). The molecular cross-talk taking place during three-way interactions requires experiments that investigate the changes in gene expression occurring in each partner involved, singly and subsequently in all possible combinations. Further, an *in situ* analysis of the compounds implicated when plants are exposed concurrently to different beneficial and/or pathogenic microorganisms could be performed.

Marra et al. (2006) studied the three-way interactions of *Trichoderma* with plant and different fungal pathogens by using a proteomic approach in order to analyze the differential proteins produced. Proteins were identified and characterized by using tryptic digestion, mass spectrometry (MS) and *in silico* analysis. Results indicated that in the plant proteome-specific PR proteins and other disease-related factors (i.e. potential resistance genes) may regulate the three-way interaction, and that the presence of the antagonist modifies quantitatively and qualitatively the plant response to a pathogen attack. In some cases, the antagonistic fungus reduced production of some defence proteins, but resulted in a higher accumulation of others. These observations suggest that the plant response to a specific BCA depends upon each of the three partners involved. On the microbial side, many differential proteins obtained from the *T. atroviride* interaction proteome showed interesting homologies to those of a fungal hydrophobin and ABC transporters. Virulence factors, like cyclophilins, were also up-regulated in the pathogen proteome during the interaction with the plant alone, as well as with the antagonist.

Gfp-tagged mutants of *T. atroviride* were used to study the in situ *Trichoderma*–plant–pathogen interaction by using different promoters of biocontrol-related genes to drive the expression of the living producer (Lu et al., 2004). In particular, induction of *Trichoderma* genes encoding for different CWDEs in the presence of the soil-borne pathogens *R. solani* and *P. ultimum* was monitored by confocal and fluorescence microscopy. During the three-way interaction the transformants were activated by the presence of the host fungal pathogen and purified colloidal chitin chitoligomers, and appeared to fluoresce during the early phases of contact. This approach allowed for the first time a direct visualization of the mycoparasitic gene expression cascade *in vivo*. The authors suggested that specific compounds released by the host cell walls were actively involved in mycoparasitism induction. In addition, the involvement of *T. atroviride* endo- and exochitinases (*nag1* and *chit42*) in the mycoparasitic process other than in the simple host hyphae degradation was also demonstrated.

Further understanding of the mechanisms operating in the interaction between plant and microbes in the soil communities could encourage development of new powerful biotechnologies, useful in the management of

fungal diseases and in the improvement of crop production yields.

2. Conclusions

The success of biocontrol agents is dependant upon the complex interactions that these beneficial microbes establish with pathogens and plants in the soil ecosystem. A better understanding of these processes and of the molecular cross-talk occurring among the participants will not only result in the application of safer and less expensive methods to protect plants and increase crop yield, but also will extend our knowledge of how a disease process develops. Recent advances in modern techniques such as proteomics and metabolomics could provide novel information about the complex tripartite interactions, in particular about the ability of *Trichoderma* to sense the environment, the plant and the microbial community. However it is clear that different approaches, i.e. genetic, molecular, biochemical and ecological, should be integrated to conduct future studies in biocontrol research and development of new technologies. In particular, a modern and more effective use of beneficial microbes such as *Trichoderma* should take into account an actual understanding of the biology and the interaction capabilities of these agents, starting with the implementation of new strain selection protocols that consider the multiple beneficial effects exerted on the colonized plant. Genetic manipulation offers novel opportunity to achieve improved biocontrol efficacy. Brunner et al. (2005) by overexpressing a glucose oxidase gene from *Aspergillus niger* in *T. atroviride* strain P1 obtained mutants able to control fungal pathogens and induce plant systemic resistance better than the wild-type strain. Finally, the information gathered by fundamental and applied studies conducted with a "wide-view" approach may allow us to overcome in the future, at least in some applications, the difficulties associated with use of living microbes. This can be done by introducing new biopesticides and biofertilizers, i.e. based on the metabolites or bioactive compounds responsible for the desired beneficial effects on crops. The application of *Trichoderma* metabolites for crop protection, such as the host defence inducers and antibiotics, may become a reality in the near future, as they can be produced cheaply in large quantities on an industrial scale, easily separated from the fungal biomass, dried and formulated for spray or drench applications. In the meantime, further experiments should be performed to better understand the mechanisms of action of *Trichoderma* secondary metabolites and their possible synergisms with other compounds used in agriculture.

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