Trichoderma – a promising plant growth stimulator and biocontrol agent

Saba H[1]*, Vibhash D[1], Manisha M[1], Prashant KS[1], Farhan H[1] and Tauseef A[1]

[1]Amity Institute of Biotechnology, Amity University, Viraj Khand-5, Gomti Nagar, Lucknow (U.P.) – India 226010, Email : saba0786@gmail.com, saba_hasan2001@yahoo.com

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Biocontrol, or Biological Control, can be defined as the use of natural organisms, or genetically modified, genes or gene products, to reduce the effects of undesirable organisms to favor organisms useful to human, such as crops, trees, animals and beneficial microorganisms. The fungus *Trichoderma*, a low cost biocontrol agent that can establish itself in different pathosystems, has moderate effects on soil balance and does not harm beneficial organisms that contribute towards pathogen's control. Fungi of the genus *Trichoderma* are soilborne, green-spored ascomycetes that are ubiquitous in nature. *Trichoderma* spp. are characterized by rapid growth, mostly bright green conidia and a repetitively branched conidiophore structure. As opportunistic plant symbionts and effective mycoparasites, numerous species of this genus have the potential to become commercial biofungicides. This biocontrol agent has no harmful effects on humans, wild life and other beneficial organisms. It is safe and effective in both natural and controlled environments that does not accumulate in the food chain. *Trichoderma* strains used as biocontrol agents can act: a) colonizing the soil and/or parts of the plant, occupying a physical space and avoiding the multiplication of the pathogens; b) producing cell wall degrading enzymes against the pathogens; c) producing antibiotics that can kill the pathogens; d) promoting the plant development and e) inducing the defensive mechanisms of the plant. The extensive studies on diverse physiological traits available and still progressing for *Trichoderma* make these fungi versatile model organisms for research on both industrial fermentations as well as natural phenomena.

Key Words – Antagonistic – Biological control – Endochitinase – Mycoparasitism

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*Corresponding author: Saba Hasan – e-mail – saba0786@gmail.com,saba_hasan2001@yahoo.com

Introduction

Worldwide traditional agricultural practices are increasingly being affected by various problems such as diseases, pests, droughts, decreased soil fertility due to use of hazardous chemical pesticides, pollution and global warming. There is thus a need for some eco-friendly biocontrol agents that may help to resolve some of these problems. Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection (Harman *et al.* 2004). Bacteria and fungi are involved in biocontrol activity, and the fungal genus *Trichoderma* plays a major role in controlling the plant diseases**.** *Trichoderma* spp. are freeliving fungi that are highly interactive in root, soil and foliar environments**.** *Trichoderma* spp. are present in nearly all agricultural soils and in other environments such as decaying wood**.** The antifungal abilities of these beneficial microbes have been known since the 1930s, and there have been extensive efforts to use them for plant disease control since then**.** *Trichoderma* is widely used as biocontrol agent against phytopathogenic fungi, and as a biofertilizer because of its ability to establish mycorriza-like association with plants. The key factor to the ecological success of this genus is the combination of very active mycoparasitic mechanisms plus effective defense strategies induced in plants**.** Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. are competition for space and nutrients, production of diffusible and/or volatile antibiotics and hydrolytic enzymes like chitinase and β-1,3- glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Kubicek *et al.* 2001). This process of mycoparasitism limits growth and activity of plant pathogenic fungi. Different species of *Trichoderma* have the potential to control soil-borne plant pathogens more effectively than chemicals and they also exhibit plant-growth promoting activity (Harman & Bjorkman 1998)**.** These fungi grow tropically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes. We do not know if most of the benefits of *Trichoderma* occur because they directly attack and control disease-causing fungi, as has long been believed, or because they have direct effects upon plants. Many recent findings suggest that plant development and biochemistry are strongly affected by *Trichoderma* strains. Specific strains of *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which in the end leads to induced systemic resistance in the entire plant**.** They can also compete with other microorganisms for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil and, more generally, compete with soil micro-organisms for nutrients and/or space. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plantpathogenic fungi. This review aims to give a broad overview on the qualities and versatility of *Trichoderma* species and to highlight intriguing findings as well as promising applications**.**

Mechanism of Action

Trichoderma in Plant Growth

Recent discoveries show that *Trichoderma spp.* are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. *Trichoderma* spp. produce extracellular β-(1,3)-glucanases, chitinases, lipases, and proteases when they are grown on cell walls of pathogenic fungi. In addition, several lines of evidence have shown that the production of some lytic enzymes is induced during the parasitic interaction between *Trichoderma* spp. and some pathogenic fungi (Haran *et al.* 1996). The chitinolytic system of *T.hrzianum* consists of five to seven distinct enzymes, depending on the strain (Haran *et al*. 1995). In the best-characterized *Trichoderma* isolate (isolate TM), the system is apparently composed of two β-(1,4)-*N*-acetylglucosaminidases (102 and 73 kDa) and four endochitinases (52, 42, 33, and 31 kDa). Different components of the chitinolytic system of *T. harzianum* probably involve complementary modes of action of the component enzymes. However, the entire system might be required for maximum efficacy (Lorito *et al.* 1993). The most interesting individual enzyme of the complex is the 42-kDa endochitinase (Ech42), which can hydrolyze in vitro *Botrytis cinerea* cell walls and inhibits spore germination and germ tube elongation of various fungi (Schirmböck *et al.* 1994). The corresponding gene (*ech42*) is strongly induced during fungus-fungus interactions and when the fungus is grown in the presence of autoclaved mycelia of several fungi or with colloidal chitin as the sole carbon source (Carsolio *et al.* 1994).

The enzymes from *Trichoderma* species that degrade fungal cell walls have been suggested to play an important role in mycoparasitic action against fungal plant pathogens. The mycoparasite *T. harzianum* produces at least two extracellular b-1,6-glucanases, among other hydrolases, when it is grown on chitin as the sole carbon source. Further characterization indicated that the enzyme by itself releases soluble sugars and produces hydrolytic thalli on yeast cell walls. When combined with other *T. harzianum* cell wall-degrading enzymes such as b-1,3-glucanases and chitinases, it hydrolyzes filamentous fungal cell walls. The enzyme acts cooperatively with the latter enzymes, inhibiting the growth of the fungi tested. Antibodies against the purified protein also indicated that the two identified b-1,6-glucanases are not immunologically related and are probably encoded by two different genes. A subtilisin-type serine proteinase induced by chitin has already been described in a mycoparasitic strain of *T. harzianum* (Geremia *et al.* 1993). Also, b-1,6-glucanases (EC3.2.1.75) have been shown to lyse yeast and fungal cell walls in filamentous fungi (Rombouts *et al.* 1976) and bacteria. Chitinases and b-1,3-glucanases have also been reported to be pathogenesis-related proteins in plants and proposed to have a major role in the defense reactions against pathogens (Broglie *et al.* 1991).

Trichoderma in Stress Tolerance

Trichoderma spp. are endophytic plant symbionts that are widely used as seed treatments to control diseases and to enhance plant growth and yield. Although some recent work has been published on their abilities to alleviate abiotic stresses, specific knowledge of mechanisms, abilities to control multiple plant stress factors, their effects on seed and seedlings is lacking. Under stress, treated seed germinated consistently faster and more uniformly than untreated seeds whether the stress was osmotic, salt, or suboptimal temperatures. The consistent response to varying stresses suggests a common mechanism through which the plant–fungus association enhances tolerance to a wide range of abiotic stresses as well as biotic stress. *Trichoderma* spp. have been known as biocontrol agents for the control of plant diseases for decades (Harman *et al.*, 2004). However, we now understand that biocontrol, in many cases, is not only related to their abilities to produce antibiotics, establish parasitic interactions, or otherwise directly affect pathogens (Howell, 2003, 2006). Instead, it is now clear that, in many cases, the beneficial fungi may induce systemic resistance that is mediated by alterations in plant gene expression (Alfano, 2007, Shoresh & Harman 2008, Shoresh *et al.*, 2010). There also are reports of enhanced plant growth as a result of the association of *Trichoderma* strains with plants but the effects, as with other plant-growth-promoting microbes (Gamalero *et al.*, 2009), are greater when plants are under suboptimal conditions or biotic, abiotic, or physiological stresses (Bae *et al.*, 2009, Mastouri Harman 2009). Several recent reports indicate that the fungi enhances tolerance to abiotic stresses during plant growth (Yildirim *et al.*, 2006), in part due to improved root growth, improvement in water-holding capacity of plants, or enhancement in nutrient uptake (i.e., potassium); whereas, in the absence of stress, plant growth may or may not be enhanced. Although molecular studies indicate greater expression of gene families involved in plant protection against abiotic stresses or oxidative damage (Alfano *et al.*, 2006, 2007, Bailey *et al.*, 2006) in *Trichoderma* spp.-treated plants, no experimental evidence has been presented correlating enhanced tolerance of plants colonized with biocontrol fungi to these changes in molecular level. These fungi are frequently applied as seed treatments, where they may improve plant stands and induce long-term improvements in plant quality (McGrew & Green, 1990). Therefore, seed treatments can induce both short-term and long-term improvements in seed and subsequent plant performance; however, very little is known about the early seed–*Trichoderma* spp. interactions. These interactions are important because (i) they can provide insights into long-term plant performance and (ii) seed–*Trichoderma* spp. interactions, if properly characterized and quantified, can provide powerful and rapid systems to examine mechanisms and physiological processes of the plant– *Trichoderma* spp. interactions. Seed respond positively to treatment with *T. harzianum* when exposed to physiological, biotic, or abiotic stresses but the beneficial fungus has little or no effect on seed not exposed to these stresses.

Treatment of seed with *T. harzianum* ameliorates a wide variety of biotic, abiotic, and physiological stresses to seed and seedlings. As far as we are aware, there has been no other systemic study focusing on the abilities of this fungus to improve seedling or growing plant performance across a variety of stressful conditions. Seed respond to *T. harzianum* very early in germination (i.e., before radicle protrusion). As mentioned, the prevailing hypotheses in this area revolve around the enhanced root growth or plant enhanced water-holding capacity due to *Trichoderma* treatment; however, if seed germination under stress is enhanced, an alternative explanation is required.

Trichoderma in Mycoparasitism

Mycoparasitism is a complex process in which a *Trichoderma* species grows chemotropically toward its host and attaches to and coils around the host hyphae, sometimes penetrating them. The mycoparasitic activity of *Trichoderma* spp. may be due to antibiosis, competition, production of cell wall-degrading enzymes (McGrew & Green 1990), or a combination of these antagonistic activities. Partial degradation of the host cell wall is normally observed in later stages of the parasitic process. The effects of cell wall-degrading enzymes on the host have been observed by using different ultrastructural and/or histochemical approaches. Some species of Trichoderma have been described as biological control agents against several fungal plant pathogens (Papavizas 1985). The degradation and further assimilation of phytopathogenic fungi, namely, mycoparasitism, has been proposed as the major mechanism accounting for the antagonistic activity of *Trichoderma* species against fungal pathogens (Che´rif & Benhamou 1990). From recent work, it appears that *Trichoderma* mycoparasitism is a complex process involving several successive steps. Initially, the mycoparasite grows directly towards its host and often coils around it or attaches to it by forming hook-like structures and apressoria. Following these interactions, *Trichoderma* spp. sometimes penetrate the host mycelium, apparently by partially degrading its cell walls (Elad *et al.*, 1984) Finally, it is assumed that *Trichoderma* spp. utilize the intracellular contents of the host. Chitin and b-1,3-glucan are the main structural components of fungal cells walls, except those from members of the class Oomycetes, which contain b-1,3-glucan and cellulose. Thus, chitinases (EC 3.2.1.14) and b-1,3-glucanases (EC 3.2.1.39), proteins secreted by *Trichoderma* spp., have been suggested as the key enzymes in the lysis of phytopathogenic fungal cell walls during mycoparasitic action (De la Cruz *et al.*, 1992, Sivan & Chet 1989). However, other cell wall-degrading enzymes, including those hydrolyzing minor polymers (proteins, b-1,6-glucans, a-1,3-glucans, etc.), may be involved in the effective and complete degradation of mycelial or conidial walls of phytopathogenic fungi by *Trichoderma* spp. *Trichoderma spp.* are used as biocontrol agents against several plant pathogenic fungi like *Rhizoctonia spp*., *Pythium* spp., *Botrytiscinerea* and *Fusarium* spp. which cause both soil-borne and leaf- or flower-borne diseases of agricultural plants. Plant disease control by *Trichoderma* is based on complex interactions between *Trichoderma*, the plant pathogen and the plant. Until now, two main components of biocontrol have been identified: direct activity of *Trichoderma* against the plant pathogen by mycoparasitism and induced systemic resistance in plants. As the mycoparasitic interaction is host-specific and not merely a contact response, it is likely that signals from the host fungus are recognized by *Trichoderma* and provoke trans-cripttion of mycoparasitism-related genes.

In the last few years examination of signalling pathways underlying *Trichoderma* biocontrol started and it was shown that heterotrimeric G-proteins and mitogen-activated protein (MAP) kinases affected biocontrol-relevant processes such as the production of hydrolytic enzymes and antifungal metabolites and the formation of infection structures. MAPK signalling was also found to be involved in induction of plant systemic resistance in *T. virens* and in the hyperosmotic stress response in *T. harzianum. Trichoderma* mycoparasitism combines processes such as nutrient competition (Chet, 1987), the secretion of antifungal metabolites (e.g. Schirmböck *et al.* 1994; Lorito *et al.* 1996) and formation of morphological changes such as coiling around the host and development of appressorium-like structures (Lu *et al.* 2004). As mycoparasitism by *Trichoderma* results in penetration of the cell wall of the host fungus and utilization of its cellular contents, hydrolytic enzymes such as chitinases, glucanases, and proteases, which are at least partially induced before direct contact with the host, play major roles in biocontrol (Hjeljord and Tronsmo, 1998). In mycoparasitic interactions between *Trichoderma* and *R. solani*, a diffusible factor released from the host is responsible for induction of *ech42* (endochitinase 42-encoding) gene transcription before physical contact (Zeilinger *et al.* 2005). Upon direct contact, lectins in the host's cell wall can induce coiling of the mycoparasite around the host hyphae. Both enzyme production and infection structure formation are induced responses triggered by molecules released from the host fungus (e.g. degradation products from its cell wall) or located on its surface (e.g. lectins) (Zeilinger *et al.* 2005).

Trichoderma in Anti Fungal Activity

Mycoparasitic *Trichoderma* species are used commercially as biological control agents against plant-pathogenic fungi such as *Rhizoctonia solani, Botrytis cinerea, Sclerotium rolfsii, Sclerotinia sclerotiorum, Pythium* spp., and *Fusarium* spp. in, among others, the United States, India, Israel, New Zealand, and Sweden as a promising alternative to chemical pesticides (Howell, 2003). The antagonistic activity of the genus *Trichoderma* to *F. solani* and *R. solani* has been widely demonstrated (Lewis et al., 1998). *Trichoderma harzianum* protected bean seedlings against pre-emergence damping off infection, reduced the disease severity and increased the plant growth in the presence of *R. solani* pathogen (Paula *et al.*, 2001). El-Kafrawy (2002) reported that *T. harzianum, T. hamatum, T. pseudoknonningii* and *T. polysporum* inhibited the radial mycelial growth of *R. solani* in vitro test from 59.6 to 78.4 %. The use of *Trichoderma* fungi in agriculture can provide numerous advantages ; 1) colonization of the root and rhizosphere of plant, 2) control of plant pathogens by different mechanisms such as parasitism, antibiosis production and induce systemic résistance , 3) improvement of the plant health by promote plant growth , and 4) stimulation of root growth (Abd-El-Khair *et al.*, 2010). *Trichoderma* spp. also are commercially marketed as biopesticides, bio-fertilizers and soil amendments.

Conclusion

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alterna-tive to the existing chemical treatment metho-ds. *Trichoderma* species have been known since the 1930s to show antifungal activity and there have been extensive efforts to use them for plant disease control since then. They have been used as biological control agents (BCAs) and their isolates have become commercially available of late. The various types of *Tricho-derma* species and their mechanism of actions mentioned above, indicate their efficacy as a potent agent of Biological Control.

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