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Trichoderma: A magical weapon against soil borne pathogens

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***Trichoderma* species occur worldwide. The members of the genus *Trichoderma* are widely used as bioagent for the control of phytopathogenic fungi in agriculture sector. *Trichoderma* not only parasites pathogen but it also enhance plant plant growth, help in the bioremediation of soil etc.Biocontrol activity of *Trichoderma* is known since 1930s.The effect of *Trichoderma* on soil borne pathogens is higher as compared to chemical fertilizers and it persists in soil for longer period after application. The members of this genus are reproducing asexually by the formation of cyanide and chlamydospore, in wild habitats they reproduce by formation of ascospores. *Trichoderma* species are well known for the production of cell wall degrading enzymes. These cell wall-degrading enzymes (CWDEs) play a major role in biocontrol mechanism. They are also widely exploited in industries as sources of enzymes.Use of biocontrol agents for reducing disease incidences provides an alternative for the chemical pesticides. *Trichoderma* species are among the most studied biocontrol agents. It has been found that there are many genes which are responsible for biocontrol activity these genes are called biocontrol genes. The sequencing of these genes should be done in order to produce miccroorganisms with superior biocontrol ability and also for the production of transgenic plant, that are resistant against plant pathogens.**

Key words:*Trichoderma,* phytopathogens, lytic enzymes, biocontrol genes.

INTRODUCTION

Use of biological pesticides is continuously increasing due to public concerns about environmental pollution, human health and soil fertility. Biological pesticides are served as an alternative to chemical pesticides. Farmers generally use chemical pesticides to control plant diseases, these chemical pesticides imparts a bad impact on environment. In Europe around 250k tones of biopesticides is consumed annually. There are two types of biocontrol agents generalists (These biocntrol agents are capable of controlling a large number of taxonomically different pathogens e.g. *Bacillus, Pseudomonas, Trichoderma*, yeast etc) and Specialist (These biocontrol agents are capable of controlling only targeted species e.g. *Agrobacterium, Aspergillus*etc).

Trichoderma species are the most commonly used biocontrol agents. They are commercially marketed as

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biopesticides, biofertilizers and growth enhancers. The various mycoparasitism mechanism employed by *Trichoderma* are, competition for food and space, secretion of cell wall degrading enzymes, secondary metabolite production, host immune response induction and plant growth promotion. *Trichoderma* based bioformulation are used in greenhouse, nursery, field, orchards and hydrophonics.*Trichoderma* based bioformulations are used for crop protection in whole world. *Trichoderma* species that are most commonly used as biocontrol agents are: *T.harzianum, T. atroviride, T. asperellum, T. polysporum, T. viride.*

Trichoderma species are also known for their biodegradation capability. *Trichoderma* species have the capability of degrading toxic compounds.

Trichoderma is highly effective on root rot, foot rot, collar rot, stem rot, damping off, wilt, blight leaf spot of crops like pulses oil seeds, *cucurbitaceous* crops (cucumber, bottle gourds, ridge gourd) *solanacceuos* crops like tomato, brinjal, chilli, capsicum etc. *Trichoderma* are also effective against sheath rot, sheath blight and bacterial leaf blight of rice.

The positive effects of *Trichoderma* on agriculture crop protection have been recognized in the whole world. Our understanding of the mechanisms of biological control employed by *Trichoderma* is continuously expanding. The molecular mechanisms of the interaction of this fungus with phytopathogen can be understood through the modern molecular techniques. Samuels (1996) provides a comprehensive review of *Trichoderma* species for enzyme production and biological control mechanisms. In *Trichoderma* species sexual reproduction is not present and is believed to be mitotic and clonal. The main problem with the nomenclature of *Trichoderma* is pleomorphisim present within the genus. In *Trichoderma* there are two stages, the sexual stage is called *Hypocrea* (telomorphic) and asexual stage is called *Trichoderma* (anamorphic). The genus is called as *Hypocrea/Trichoderma* (Druzhinina et al., 2011).The genetic diversity within the genus *Trichoderma* is very high, thus there must be some technique through which we can identify the *Trichoderma* species. The necessary characters which should be present in an effective biococntrol agent are: Good lytic enzyme producer, increased plant systemic resistance, plant growth enhancer, good secondary metabolite production, pollutant degradation, good CFU maintenance in formulation. By gaining the knowledge of desirable characters new strains can be designed and developed. In case of *Trichoderma*protoplast fusion is the technique through which we can develop hybrid strains.

Trichoderma species are excellent producers of lytic enzymes like chitinase, glucanase, cellulases and xylanase, which lyase the fungal cell wall. Besides being attacking directly it promotes plant growth and induce plant resistance. *Trichoderma* species produce a variety of secondary metabolites. The *Trichoderma*species release antibiotics and other chemicals that are harmful to pathogens and inhibit growth (antibiosis).The potential use of the *Trichoderma* species (Table 1) as a biocontrol agent was suggested more than 70 years ago by Weindling (1932) who was first to demonstrate the parasitic activity of a member of this genus against soil borne fungal or bacterial pathogens.There are many other crops for which *Trichoderma* is used.

TAXONOMIC HISTORY

Although the genus *Trichoderma* has been known since the 19th century. Its association with *Hypocrea*Fr. was discovered by the Tulasne brothers in 1865; its taxonomy has remained obscure until recent decades. Bisby (1939) thought that the morphological variation could be ascribed to a single species, *T. viride*. The first serious attempt to morphologically distinguish species, or rather “species aggregate”, was made by Rifai (1969). Some new species were subsequently described by were keyed out by Domsch et al.(1980). Teleomorph connections were established by means of ascospore isolates by Dingley (1957), and by Webster and coworkers Webster andRifai (1969). In Japan, Doi (1969; 1972), studied a number of telemorphs and described them with cultural and anamorph characters, but no cultures were preserved from that study. After this no morphological differentiation was given by Doi(1979; 1986). Bissett (1984; 1991a, b, c; 1992) gave a detailed description of the morphological studies, who distinguished about 21 taxa. These studies have shown the delimitation of biological species is extremely difficult in this genus on morphological grounds alone.

Apart from morphological studies there are many other methods that are used in the taxonomy, such as study of secondary metabolites, this has shown a great diversity in this genus (Okuda et al., 1982). Physiological features that are detected by the microtiter plate assay are the useful tools that are used for identification. Isoenzyme profiles are also used for taxonomic classification (Leuchtmann et al., 1996; Samuels et al., 1994). In modern era molecular techniques such as sequences of its region of ribosomal DNA and fingerprinting techniques provides the finest resolution of taxonomic entities(Fujimori and Okuda, 1994; Meyer et al., 1992; Muthumeenakshi et al., 1994).

BIOCONTROL GENES OF *TRICHODERMA*

Trichoderma is widely used for the control of many soil borne plant pathogens (Table 2). *Trichoderma* species are the efficient producer of cell wall degrading enzymes (Srivastava et al., 2014b), some of these enzymes are of commercial importance. Many research workers have proved that *Trichoderma* species possess some

Table 1. *Trichoderma* species and its uses against different plant pathogens.

Plant	Causative agent	*Trichoderma* species used
Vignamungo (Black gram) (Raghuchander et al., 1997; Dubey et al., 2001; Mishra et al., 2011)	Macrophominaphaseolina, alternate Alternaria	*T. viride, T. harzianum*
Solanummelongena L.(Brinjal) (Jadon, 2009, Balaji and Ahir, 2011)	*Fusariumsolani F.oxysporum f. sp*	*T. viride, T. harzianum*
Cicerarietinum (Chickpea) (Mukherjee et al., 1997; Haware et al., 1999; Pandey, 2003; Poddar et al., 2004)	*F. oxysporum, R. solani, A. niger* *Chaetomium* sp, *S. rolfsii, Penicillum* spp. *M. phaseolina*	*T. harzianum, T. viride*
Capsicum annum L (Chilli), (Rini and Sulochana, 2006, Kapoor, 2008, Vasanthakumari and Shivanna 2013)	*S. rolfsii, F. oxysporum, Pythiumspp, R. solanipseudokoningii 2013*	*T. viride, T. harzianum*
Cocosnucifera L (Coconut) (Karthikeyan et al., 2006)	*Ganodermalucidum*	*T. harzianum, T. viride*
Coffeaarabica L. (Coffee); (Deb and Dutta,1999)	*Phomopsisthaeae, Glomerellacingulata*	*T. harzianum*
Vignasinensis (Cowpea) (Pan and Das, 2011)	*R. solani*	*T. harzianum*
Arachishypogaea L.(Groundnut) (Biswasand Sen, 2000; Kishore et al., 2001; Rakholiya et al., 2010; Bagwan, 2011; Sreedevi et al., 2011; 2012)	*Thievaliopsisbasicola, S. rolfsii Sacc, A. niger, R. solani, P aphanidermatum, M. phaseolina*	*T. harzianum T. viride T longibrachiatum*
Agaricusbisporus (Mushroom) (Rawal et al., 2013)	*Rhizopusstolonifer, Coprinopsiskimurae, P. glabrum, F. oxysporum*	*T. viride*
Cajanuscajan (Pigeon pea) (Hukma and Pandey, 2011)	*F. udum*	*T. viride T. harzianum*
Solanumlycopersicum (Tomato) (Sreenivasaprasad, 1999; Dutta and Das, 2002; Jayaraj, 2006,)	*F. oxysporum f. sp. lycopersici, P. aphanidermatum, R. solani, S. rolfsii*	*T. harzianum T. viride T longibrachiatum, T. virens*
Capsicum annum L (Capsicum) (Kapoor, 2008)	Alternariaalternata	*T. viride T. harzianum*
Brassica oleracea (Cauliflowe) (Sharma et al., 2004; 2005; Ahuja et al., 2012)	*R. solani, P. aphanidermatum*	*T. viride T. harzianum*
Citrus (Kalita et al., 1996; Singh et al., 2000	*F. solani*	*T. viride T. harzianum*
Gossypiumhirsutum (Cotton) (Sreenivasaprasad, 1990; Gaur et al., 2005)	*R. solani, S. rolfsii, P. aphanidermatum*	*T. viride T. harzianum*
Zingiberofficinale (Ginger)(Gupta et al., 2010)	*P. aphanidermatum*	*T. harzianum*
Sesamumindicum L (Sesame) (Tamimi and Hadvan, 1985; Sankar and Jeyarajan, 1996a,b; Jeyalakshmi, 2013)	*A. flavus, Curvularialunata, P. notatum, P. chrysogenum, F. moniliforme, F. oxysporium , R. nigricans, M. phaseolina*	*T. viride T. harzianum*

Table 2. Some biocontrol genes of *Trichoderma* and their function.

Gene	Source organism	Function
Tvsp1	*T. virens*	This gene encodes for serine protease. *Rhizoctonia solani which* affects the cotton seedlings has been controlled biologically by serine protease.
*tri*5	*T. harzianum*	This gene is responsible for the synthesis of trichothecene which inhibits the protein and DNA synthesis in the cells of the pathogens and inhibits their growth.
*TgaA*and *TgaB*	*T. virens*,	This gene exhibits antagonist activity against *R. solani*and *Sclerotiumrolfsii*
ThPG1	*T. harzianum*	This gene encodes for endopoly-galacturonase. This enzyme is involved in the cell wall degradation of the pathogens like *R. solani*and *P. ultimum*
Th-Chit	*T. harzianum*	This gene is responsible for the antifungal activity in transgenic tobacco plant.
tri5	*T. brevicompactum*	This gene helps in the production of *Trichoderm* in which shows antifungal activity against *S. cerevisiae, Kluyveromycesmarxianus, Candida albicans, C. glabrata, C. tropicalis*and *Aspergillus fumigates*
erg1	*T. harzianum*	This gene encodes an enzyme named squalene peroxidase, which helps in the synthesis of ergosterol and silencing of this gene provides resistance to terbinafine, an antifungal compound
TvGST	*T. virens*	This gene is responsible for cadmium tolerance
Thkel1	*T. harzianum*	This gene codes for putative kelch-repeat protein which helps in regulating the glucosidase activity and enhances tolerance to salt and osmotic stresses in *Arabidopsis thaliana* plants
egl1.	*T. longibrachiatum*	This gene showed biocontrol activity against *P. ultimum*in damping-off of cucumber
qid74	*T. harzianum*CECT 2413	This gene plays a significant role in cell protection and provide adherence to hydrophobic surfaces that helps the fungus in mycoparasitic activity against *R. solani*pathogen
Taabc2	*T. atroviride*	This gene has a significant role in ATP binding cassette (ABC) transporter in cell membrane pump that helps in the mycoparasitic activity
tac1	*T. virens*	This gene has its role in mycoparasitic activity against *R. solani*and *P. ultimum*
TrCCD1	*T. reesei*	This gene is involved in carotenoid metabolism that helps in the development of conidiospores and hyphal growth in *T. reesei*

Source:Srivastava et al. (2014b).

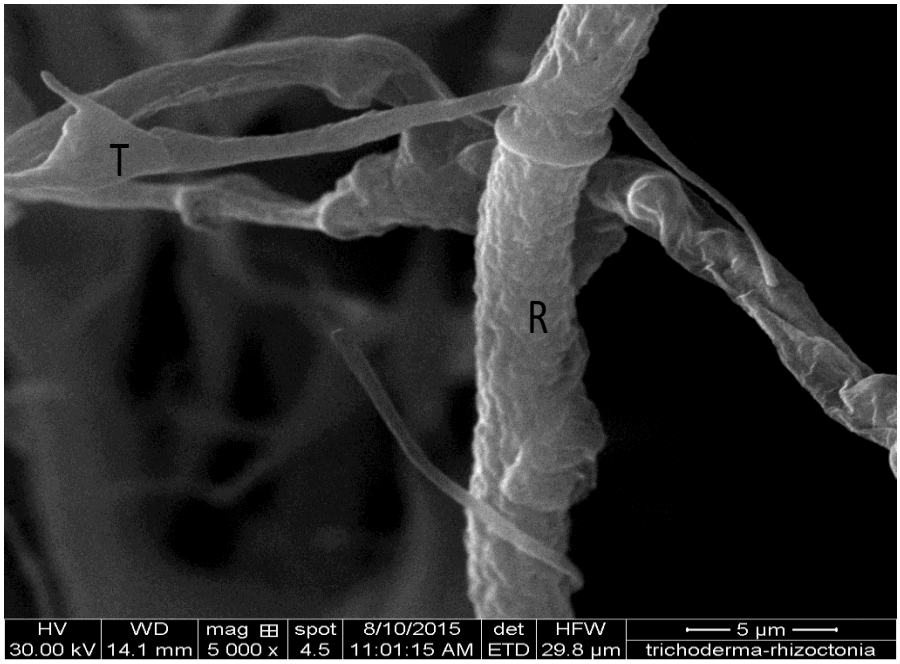

Figure 1. SEM analysis of coiling and hyphal growth depression of *Rhizoctonia bataticola* (R) *by T. harzianum* (T).

biocontrol genes that can be isolated and cloned for commercial large scale production (Massart and Jijakli, 2007).

Kuc (2001) has proven that some genes, providing resistance to abiotic and biotic stress are present in *Trichoderma*. Mycoparasistism, antibiosis and competition for the nutrients are the main strategies employed by *Trichoderma* for the phytopathogenic action (Janisiewicz and Korsten, 2002). Among the different species tested *T. herzianum* was found to be the most promising strain (Gao et al., 2002).

GENOMICS OF TRICHODERMA

The genome size of filamentous fungi is very small that is about 25 to 50 Mb. With the advancement of pulse field gel electrophoresis karyotyping of the filamentous fungi is possible. Karyotyping is helpful in the detection and translocations and variations in chromosome numbers. Through PFGE we separated chromosomal DNA from *Trichoderma* (Gilly and Sands, 1991; Hayes et al., 1993; Herrera-Estrella et al., 1993; Mäntylä et al., 1992). The expected genome size and chromosome number of *Trichoderma* ranges from 31 to 39 MB and from 3 to 7, respectively. From the data obtained through DNA homology it was found that *T. herzianum*and *T. veins* are closely related, and it was concluded that they may have the same phylogenetic origin (Herrera-Estrella et al., 1993). Mäntylä et al., (1992) determined molecular karyotypes of strains of *T. reesei* that had undergone mutagenesis and screening for the hyperproduction ofcellulase enzyme. The authors found that extensive alteration in the genome organization of these strains occurred.

The first member of the genus sequenced was *T. reesei*. *T. reesei* is the first choice, because the genome size of this organism is very small (33Mb) and it has seven chromosomes. Fungal genomics laboratory of NCSU has EST, cDNA collection and BAC libraries for academic researchers. DNA mediated transformations and gene protocols have been developed for the genomic study of *Trichoderma.*

Trichoderma and phytopathogen control

The use of *Trichoderma* as a biocontrol agent for the control of phytopathogen is an environmental friendly process. However, more detailed information about the mechanism of action of these biological agents is needed (Cortes, 1998). When *Rhizoctonia* comes in contact with *Trichoderma herzianum*, hyphae of the *T. herzianum* start to coil around *Rhizoctonia* (Chet, 1987).). It has been found that there are some lectins, secreted by *Rhizocotonia, Sclerotium* and other phytopathogenic fungi which stimulate *Trichoderma* to coil around pathogen hyphae (Figure 1). *Trichoderma* species secrete extracellular enzymes that aid in the phytopathogenic activity. Weindling (1934) reported that a strain of *Trichoderma*, *T. lignorum* secretes gliotoxin that is harmful for both *R. soleni* and *Sclerotium* americana

(Chakravarthy et al., 2011). It has been found that chitinolytic system of *Trichoderma* has six different enzymes two of which are classified as acetyl glucosamine and the rest four as endochitinase (Haran et al., 1996).

Mycoparasitism can be defined as the direct attack of one fungi over other (Fand et al.,2013). The process of mycoparasitic action involves recognition, attachment and finally killing of the fungi. CWDEs play a major role in the mycoparasitism action. Mycoparasitism action begins with the secretion of chitinase enzyme, which degrades the cell wall of the fungus. As soon as cell wall of the phytopathogen degrades it enters into the lumen of the pathogen and kills that pathogen. In *Trichoderma* there is an evidence for the participation of G α unit in coiling.

The heterometric G protein signaling is basically comprise of three parts, a G protein-coupled receptor(GPCR), a heterotrimeric G protein (α, β, γ subunits), and an effector (Neer, 1995). Various fungal genomes are available nowadays and comparative genomics pointed out that receptors can be classified into nine groups (Lafon et al., 2006). Preliminary investigations of the *Trichoderma reesei* (http:// genome.jgi-psf.org/Trire2/Trire2.home.html) and *T. atroviride* genomes revealed 16 putative proteins with 7-transmembrane domains, well distributed over all nine receptor classes. Highly conserved heterotrimeric G-proteins act as signal transducers that couple cell surface receptors to cytoplasmic effector proteins. G-protein α subunits can be classified into three major subgroups (Bölker, 1998). Further characterization of the tga1 mutant showed that this G-protein α subunit affects processes like vegetative growth, production of antifungal metabolites, and chitinase formation (Reithner et al., 2005), which are at least partially involved in *Trichoderma*biocontrol. In liquid culture the tga1 mutant produced strongly decreased chitinase activities and showed a reduced transcription of the nag1 (N-acetyl-glucosaminidase encoding) and ech42 (endochitinase 42-encoding) genes (Reithner et al., 2005). In antagonistic assays, the tga1 mutant was unable to overgrow and lyse host fungi such as *R. solani,B. cinerea*, and *S. sclerotiorum*, although infection structure formation was unaffected; nevertheless, it displayed an enhanced growth inhibition of the host fungi by over-producing and secreting low molecular weight metabolites. In contrast to the role of Tga1 in influencing growth and conidiation in *T. atroviride*, its homologue TgaA did not affect these properties in *Trichoderma virens*. tgaA mutants grew normally and sporulated like the wild type, but had a reduced ability to colonize *S. rolfsii*sclerotia, whereas they were fully pathogenic against *R*. *solani* (Mukherjee et al., 2004). No such host specificity could be observed in the *T. atroviride* tga1 mutant. Mutants of *T. virens* lacking the TgaB protein(belonging to subgroup II Gα subunits) did not show major phenotypic defects: They grew and sporulate like the wild type and biocontrol against *R. solani* and sclerotia of *S. sclerotiorum* was unaffected (Mukherjee et al., 2004).

MECHANISMS OF PLANT-DISEASE CONTROL BY *TRICHODERMA*

When *Trichoderma* spores are added into the soil they colonize the root surface and form a zone of interaction into which *Trichoderma* species release bioactive compounds. These bioactive compounds enhance plant resistance.The *Trichoderma* species produce CWDEs that degrade the cell walls of pathogen, it also produces antibiotics, it starts coiling around the pathogen hyphae.

In recent times excessive use of chemical pesticides has pose a threat on the environment.*Trichoderma* based biocontrol agents have better ability to promote plant defense response, promote plant growth and soil remediation etc. *Trichoderma* species have gained wide acceptance as effective biocontrol agents against several commercial phytopathogens (Sitansu and Amrita 2011). Micropropagules of *Trichoderma* spp. in the form of conidia are preferred over chlamydospores and mycelia biomass because of the viability and stability in field application (Rosaneet al., 2008).

BENEFITS OF TRICHODERMA

1. *Trichoderma* is extensively used for post-harvest disease control. It has been found effective against Fusarium*, Phytopthara, Scelerot* etc.
2. *Trichoderma* strains have the ability to solubilize phosphates, thus they act as plant growth promoting Rhizobacteria.
3. There are many biocontrol genes are present in *Trichoderma*. Introduction of endochitinase gene from *Trichoderma* into plants such as tobacco and potato plants have increased their resistance to fungal growth.
4. *Trichoderma* strains also play an important role in the bioremediation of soil thatare contaminated with pesticides and herbicides. They have the ability to degrade a wide range of insecticides, organochlorines, organophosphates and carbonates.
5. *Trichoderma* is environmentally friendly.
6.*Trichoderma*species are efficient producer of cellulase and other enzymes that degrade complex polysacchharides. Cellulase from *Trichoderma* are used for the biostoning of denims, to give it a stone washed appearance.
7.The extracellular enzymes obtained from *Trichoderma* are also used to increase the digestibility hemicelluloses used in poultry feed.
8. *Trichoderma* is compatible with organic manures and with other biofertilizers like *Rhizobium,Azospirilium*, *Bacillus subtilis* etc. It can be easily applied to seeds

treated with metalaxyl and thiram but not with mercurials (Gangwar and Sharma, 2013).

LIMITATIONS

1. We should not apply chemical fungicides after application of*Trichoderma* for 4 to 5 days.
2. Trichoderma should not be used in dry fields, as it requires moisture for its survival.
3. Seeds treated with *Trichoderma* should not be dry in direct sunlight**.**

CONCLUSION AND FUTURE RESEARCH

Trichoderma is a promising candidate for the biological control of plant pathogenic fungi.Data obtained from the past studies have provided many clues for future studies. Data obtained by researchers clearly shows that this fungus can be efficiently used as biocontrol agent. The genes present in the fungi have the ability to enhance host plant's resistance against phytopathogenic fungi.

Conflict of Interests

The authors have not declared any conflict of interests.

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