

Review

Biocontrol genes from *Trichoderma* species: A review

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Accepted 25 November, 2011

In the world, the traditional agricultural practices are getting affected by various problems such as disease, pest, drought, decreased soil fertility due to use of hazardous chemical pesticides, pollution and global warming. As a result, there is a need for some eco-friendly biocontrol agents that help in resolving the previous mentioned problems. The various types of biological control agents such as bacteria and fungi are involved in biocontrol activity. Among them, fungal genus *Trichoderma* plays a major role in controlling the plant diseases. The species of *Trichoderma* are known to produce different kinds of enzymes which have a significant role in biocontrol activity like cell wall degradation, biotic and abiotic stress tolerance, hyphal growth, antagonistic activity against plant pathogens. By the advance techniques laid in the molecular biology, we can easily isolate, characterize, clone, sequence and express the functions of these genes and can study their functions and role in the biocontrol mechanism. This review article explains about the role, and functions of some major biocontrol genes present in the *Trichoderma* species viz., *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma atroviride*, *Trichoderma reesei*, *Trichoderma hamatum* and *Trichoderma longibrachiatum*.

Key words: *Trichoderma* spp., genes, cloning, transformation, expression.

INTRODUCTION

The species of *Trichoderma* are well known for their biocontrol activity against many plant pathogens that cause major problems in the current agricultural scenario. *Trichoderma* species are known for their production of cell wall degrading enzymes which can be used for commercial productions. All living organisms are made up of genes that code for a protein which performs the particular functions. Genes play a major role in the biocontrol process by regulating some signals and lead to the secretion of some enzymes or proteins that help in the degradation of the pathogens and hence they are known as biocontrol genes. Increased expression of the genes helps in enhanced biocontrol activity which helps in promoting the plant growth and prevents the plant from pathogen attack. So the biocontrol genes can be cloned and produced in large amounts for commercial applications (Massart and Jijakli, 2007).

Some genes of *Trichoderma* species can be used to provide resistance to the biotic and abiotic stresses such

as salt, heat and drought (Kuc, 2001). Before using as a commercial product, the biocontrol mechanism of a particular species should be well known (Grondona et al., 1997). The major biocontrol process involves antibiosis, providing plant nutrition and mycoparasitism (Janisiewicz and Korsten, 2002).

Trichoderma species are used widely as biocontrol agents because they have more benefits on plant growth such as promoting plant growth, increasing the nutrient uptake from the soil, and decreasing the activity of the soil borne pathogens that ultimately affect the growth of the plant (Harman et al., 2004). Among the various species of the *Trichoderma*, *Trichoderma harzianum* is considered to be the most effective biocontrol agent (Gao et al., 2002).

BIOCONTROL GENES AND THEIR FUNCTIONS FROM *TRICHODERMA* SPECIES

The genus *Trichoderma* acts as a biocontrol agent due to its feasible character in fighting against the pathogens. Some major kinds of biocontrol genes that can be easily

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isolated, cloned and characterized are protease, chitinase, glucanase, tubulins, proteinase, xylanase, mono-oxygenase, galacturonase, cell adhesion proteins and stress tolerant genes. These genes have their unique functions in the biocontrol mechanism such as cell wall degradation, hyphal growth, stress tolerance, and parasitic activity. Tubulins are structural proteins made of microtubules and they help in studying the cell wall composition of the pathogens (Li et al., 2010). Chitinase helps in the breakdown of the glycosidic bonds. Glucose oxidase catalyses D-glucose to D-glucono-1,5-lactone and hydrogen peroxide are known to have antifungal effect (Ciliento et al., 2004). Xylanase helps in breaking hemicellulose a major component of plant cell walls (Figure 1).

FUNCTIONS OF BIOCONTROL GENES

Cell wall degradation

From *Trichoderma virens*, a gene named *tvsp1* encoding for serine protease was cloned successfully and its function was analyzed. Serine protease has an important role in pathogenesis or biocontrol activity against *Rhizoctonia solani* which affects the cotton seedlings. The gene *tvsp1* was expressed in *Escherichia coli* and cloned using pET-30 vector. Thus, serine protease helps in degrading the fungal cell wall (Pozo et al., 2004).

In *T. harzianum* trichodiene synthase gene *tr5* was isolated and characterized. This *tr5* gene was responsible for the synthesis of the enzyme trichothecene which inhibits the protein and DNA synthesis in the cells of the pathogens and inhibits their growth. The trichothecene shows phytotoxic activity against *Fusarium* species. The gene *tr5* was isolated and by designing of specific primers. The sequence was inserted into pGEM-T vector, cloned and expressed.

The presence of *tr5* gene was confirmed by screening with other *Trichoderma* isolates (Gallo et al., 2004). The expression of gene *tag83* which encodes cell wall degrading enzyme exo- β -1,3-glucanase was isolated from *Trichoderma asperellum* and characterized. The expression analysis of this gene was studied using real time and reverse transcription-polymerase chain reaction (RT-PCR). The enzyme activity of glucanase was studied by comparing with various types of carbon sources like starch, cellulose, chitin, chitosan and cell walls of *R. solani*. The expression of *tag83* gene with *R. solani* showed that glucanase enzyme exhibits parasitic activity against pathogens (Marcello et al., 2010).

T. virens transformants expressed two different kinds of β -1,3 and β -1,6 glucanase genes viz., *TvBgn2* and *TvBgn3*. These genes secrete cell wall degrading enzyme that helps in the biocontrol activity. *T. virens* GV29.8 wild type and double over expression (DOE) transformant strains were used to detect the enzyme activity against

pathogens like *R. solani*, *Pythium ultimum* and *Rhizopus oryzae* (Djonovic et al., 2007).

A gene, *gluc78* which codes for an antifungal glucan 1,3- β -glucosidase was isolated, cloned and sequenced from *Trichoderma atroviride*. This gene has its significance in the cell wall degradation of the pathogens. The gene *gluc78* was cloned in pGEM-T vector and the expression analysis was done against pathogens such as *R. solani* and *P. ultimum* (Donzelli et al., 2001). From *T. harzianum*, a glucose repressor gene *crel* was isolated and characterized. This gene causes the repression of cellulase and xylanase encoding genes. Cellulase and xylanase are the major type of enzymes that involve in the cell wall degradation of the pathogens. The gene was cloned using pTZ57R/T plasmid vector and transformed into *E. coli* DH 10B and the role of *crel* gene in cellulase and xylanase expression was studied (Saadia et al., 2008).

β -Tubulins are structural components of most cells and they interact with benzimidazole fungicides, and play a major role in biocontrol process. This β -tubulin gene was isolated and characterized from *T. harzianum*. The β -tubulin gene was amplified by PCR, the coding regions and the flanking sequences were identified using inverse and nested PCR. The sequences were analyzed for the presence of motifs for the expression of the gene. The three dimensional model of β -tubulin gene was done by Swiss-model automated comparative protein modeling server (Li and Yang, 2007). From *T. virens*, a gene, Sm1 a cysteine-rich protein was isolated and expressed. It shows defense activity against diseases in dicot and monocot plants (Buensanteai et al., 2010).

Serine proteases play a key role in the fungal biology and involves in biocontrol activity. From *T. harzianum* a novel serine protease gene named SL41 has been cloned and expressed successfully in *Saccharomyces cerevisiae*. The cDNA of SL41 gene was sequenced and it was cloned in pMD18-T vector and the yields were inserted into *E. coli* DH5- α . Thus, serine proteases were cloned and characterized (Liu et al., 2009).

The gene, *ThPG1* which encodes for endopolygalacturonase was isolated from *T. harzianum* and characterized. This enzyme involves in the cell wall degradation of the pathogens like *R. solani* and *P. ultimum* and helps in the plant beneficial interactions. The expression study of this gene was studied by comparing the wild and mutant type strains.

The full length cDNA clone of *ThPG1* gene was obtained by polymerase chain reaction and was cloned in pSIL-pG1 vector. The phylogenetic relationship was obtained by Neighbor-joining (NJ) tree method (MoranDiez et al., 2009). A gene, *Tv6Gal* which codes for endo- β -(1 \rightarrow 6)-galactanase gene was isolated from *T. viride*, cloned and expressed in *E. coli*. Galactanase enzymes belong to the family of arabinogalactan proteins that involve in cell-cell adhesion, cell expansion and cell death. The cDNA clone of the gene *Tv6Gal* was done by

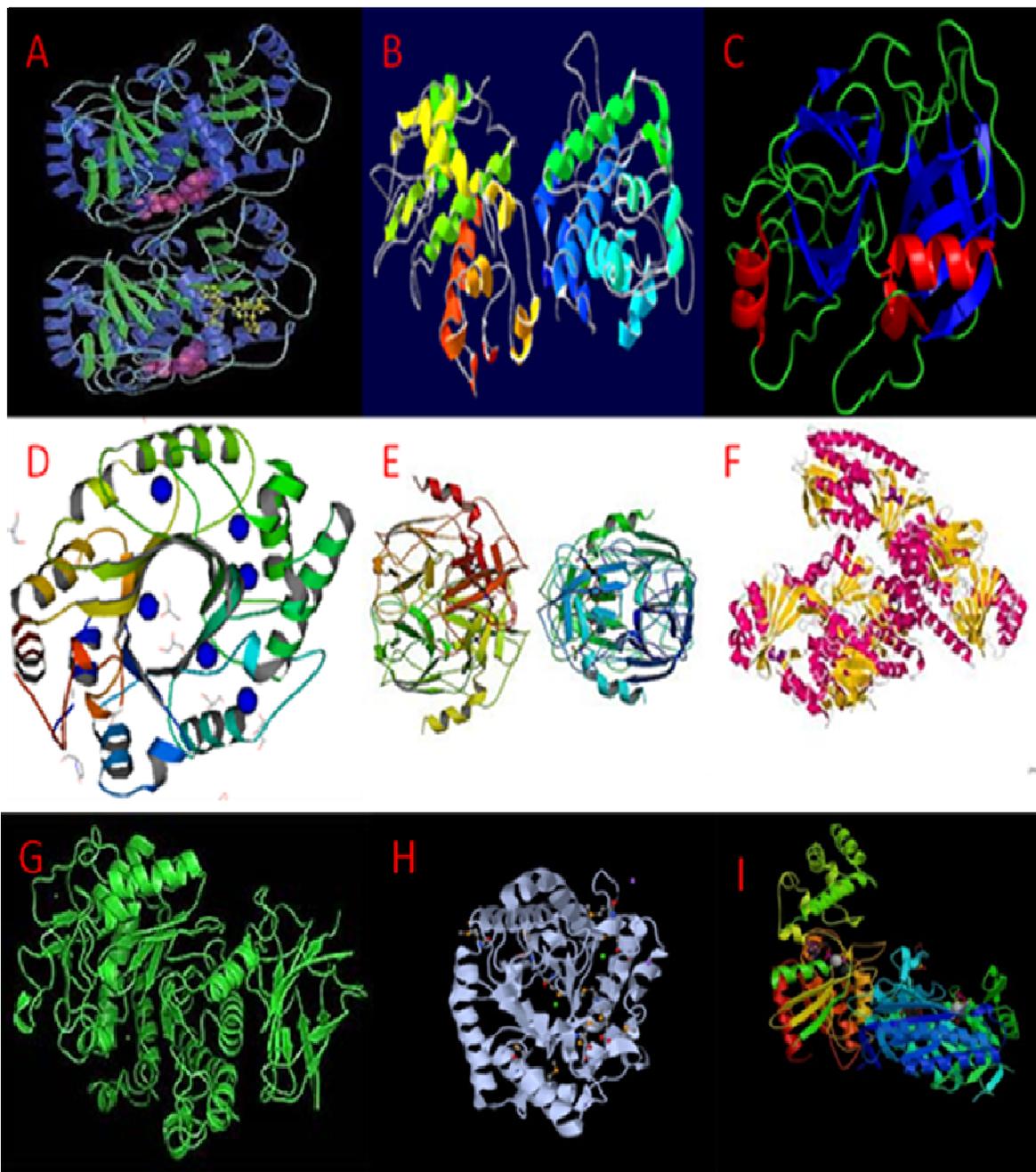


Figure 1. Structure of biocontrol genes: **A)** tubulin, **B)** chitin, **C)** protease, **D)** xylanase, **E)** proteinase, **F)** monooxygenase, **G)** β -endogalactanase, **H)** β -endoglucanase, and **I)** adenylate-Cyclase. (www.google.co.in/images). From *T.harzianum* gene encoding for endochitinase and β -tubulin has been cloned and characterized successfully using pGEM-T vector ongoing work (senior author).

RT-PCR, cloned in pGEM-T vector and expressed in *E. coli* (Kotake et al., 2004).

Xylanase producing *Trichoderma* strain SY was isolated from the soil. The gene coding for xylanase, *Xyl* was cloned by RT-PCR. *Xyl* was highly expressed when it was grown in cellulose as an only source of carbon. The full length cDNA of *Xyl* was amplified by PCR and

cloned in pGEM-T vector. The cloned gene was expressed in *E. coli* and the proteins were analyzed using sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) (Min et al., 2002). From *T. virans*, the g-protein α subunits genes, *TgaA* and *TgaB* were cloned and characterized. This gene exhibits antagonist activity against *R. solani* and *Sclerotium rolfsii*

(Mukherjee et al., 2004) (Table 1).

Biotic and abiotic stress tolerances

Species of *Trichoderma* helps the plant to survive in the abiotic stress conditions. From *T. harzianum* T34 isolate, *hsp70* gene was cloned and characterized. This gene helps in increasing the fungal resistance to heat and other stresses such as salt tolerance, osmotic and oxidative tolerances. The protein sequences were analyzed using DNASTAR package and aligned using CLUSTAL X algorithm. The *hsp70* gene was cloned in pGEM-T vector and expressed in different isolates of *T. harzianum* (ManteroBarrientos et al., 2008).

From the fungus *T. harzianum*, a gene named *Thkel1* was isolated and characterized. 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. The vector used for cloning was pSIL-KEL and was transformed to *T. harzianum*. The expression of this gene was studied by growing the fungal at various biotic and abiotic stress conditions (Hermosa et al., 2011). From *T. virens* glutathione transferase gene *TvGST* was cloned. When transgenic plant expresses this gene against different concentrations of cadmium, it shows tolerance to cadmium accumulation in plants. Thus it acts as cadmium tolerant gene (Dixit et al., 2011).

Mycoparasitism

From 31 isolates of *T. harzianum*, five have been selected namely (T 30, 31, 32, 57 and 78) and from them genes encoding for *N-acetyl-β-D-glucosaminidase* (*exc1* and *exc2*), chitinase (*chit42* and *chit33*), protease (*prb1*) and β-glucanase (*bgn 13.1*) were cloned and expressed. These genes play a major role in the mycoparasitic activity against the pathogens especially *Fusarium oxysporum*. The expressions of these genes that codes for these enzymes were determined by RT-PCR and their effects against the pathogens were tested by dual plate assay (LopezMondejar et al., 2011). In *T. virens*, an adenylate-cyclase encoding gene named *tac1* gene was isolated and cloned. This gene has its role in mycoparasitic activity against *R. solani* and *P. ultimum* (Mukherjee et al., 2007). *ThPTR2* a di or tri peptide transporter gene isolated from *T. harzianum* CECT 2413 has a significant role in the mycoparasitic activity against *Botrytis cinerea*.

The cDNA of *ThPTR2* gene was obtained through reverse transcript polymerase chain reaction, transferred into pBRC43 plasmid, cloned and expressed. The sequences were aligned using CLUSTAL-W algorithm and protein binding motifs were discovered. The mycoparasitic expression of the *ThPTR2* gene was analyzed

by dual culture assay (Vizcaino et al., 2006). The gene, *qid74* isolated from *T. harzianum* CECT 2413 was found to play a significant role in cell protection and provide adherence to hydrophobic surfaces that helps the fungus in mycoparasitic activity against *R. solani* pathogen. The function of this gene was studied by comparing the expression of genes in wild type transformants and disruptants. The results showed that *qid74* gene was responsible for adhesion to the hydrophobic surfaces of the pathogenic fungi and helps in the antagonistic activity (Rosado et al., 2007).

A gene named, *Taabc2* was cloned from *T. atroviride* and characterized. This gene has a significant role in ATP binding cassette (ABC) transporter in cell membrane pump that helps in the mycoparasitic activity. The expression of this gene was found to be more when they uptake the nutrients from the pathogenic fungi. The gene was cloned using pGEM-T vector, expression of the genes were analyzed using RT-PCR.

The antagonist activity against pathogens such as *R. solani*, *B. cinerea*, and *P. ultimum* was done by dual culture plate assay with *T. atroviride* wild and mutant type strains (Ruocco et al., 2009). From *T. harzianum* genes encoding for proteinase *prb1* and endochitinase *ech42* were isolated and characterized. These genes involved in the mycoparasitic activity against *R. solani* and *S. rolsfii*. For the production of these enzymes, the genes were induced by lectin-carbohydrate interaction a diffusible factor. This factor regulates the production of proteinase and endochitinase which helps in the mycoparasitism (Cortes et al., 1998).

In *Trichoderma hamatum* the expression of mycoparasitism genes, namely chitinase *chit42* and proteinase *prb1* were analyzed. The expressions of these genes were analyzed by confrontation assay against the plant pathogen *Sclerotinia sclerotiorum*. During sequence analysis the presence of motifs was discovered and that helps in the regular expression of the genes that enhances the parasitic activity against pathogens (Steyaert et al., 2004).

T. longibrachiatum transformants showed over expression of β-1,4-endoglucanase gene *egl1*. This gene showed biocontrol activity against *P. ultimum* in damping-off of cucumber. The *egl1* gene, coding for endoglucanase was isolated from *T. longibrachiatum*, cloned and expressed in *Saccharomyces cerevisiae*. The expression of the gene was compared with the wild type and transformed strains. The results showed that the over expression of *egl1* gene showed good biocontrol activity (Migheli et al., 1998). TmkA, mitogen activated protein kinase from *T. Virens* is known to cause mycoparasitic activity against *R. solani* and *S. rolsfii* (Mukherjee et al., 2003).

Antifungal activity

A transcription factor gene named *Thctf1* was isolated

Table 1. List of biocontrol genes isolated from different *Trichoderma* species and their functions.

S/N	Name of the isolate and biocontrol gene	Function	Strain identification by DNA sequence analysis with accession numbers	Effect of the gene in biocontrol	Author	Year
1	<i>T.harzianum</i> strain IMI206040 (proteinase <i>prb1</i> and endochitinase (<i>ech42</i> genes))	Parasitic activity against <i>Sclerotium rolfsii</i> and <i>Rhizoctonia solani</i> .	Accession numbers not available	Expression of this gene helps in regulation of hydrolytic enzymes.	Cortes et al.	1998
2	<i>T.longibrachiatum</i> wild type strain CECT2606 (β -1,4-endoglucanase gene, <i>egl1</i>)	Biocontrol activity against <i>Pythium ultimum</i> on cucumber.	Accession numbers not available	Shows enhanced biocontrol activity.	Migheli et al.	1998
3	<i>T.harzianum</i> strain P1 74058 (<i>ech42</i> gene)	Biocontrol activity against <i>Botrytis cinerea</i> and <i>R. solani</i>	Accession numbers not available	Disruption of this gene affects the biocontrol activity.	Woo et al.	1999
4	<i>T.atroviride</i> strain P1 (ATCC 74058) (1,3- β -glucosidase gene, <i>gluc78</i>)	Cell wall degradation of pathogens <i>Pythium</i> and <i>Phytophthora</i> .	GenBank AF253421	Exhibits moderate biocontrol activity.	Donzelli et al.	2001
5	<i>Trichoderma</i> strain SY (Xylanase gene <i>Xyl</i>)	Helps in breakdown of hemicellulose.	GenBank AY156910	Only gene isolation	Min et al.	2002
6	<i>T.virens</i> strain IMI 304061 (<i>TmkA</i> Mitogen Activated Protein kinase gene)	Biocontrol activity against pathogens like <i>S. rolfsii</i> and <i>R. solani</i> .	GenBank AY141978	This gene represses the conidial formation of <i>R.solani</i> .	Mukherjee et al.	2003
7	<i>T.harzianum</i> strain ATCC 90237 (trichodiene synthase <i>tri5</i> gene)	A toxic secondary metabolite which is responsible for inhibiting DNA or protein synthesis, and enhances virulence against <i>Fusarium</i> spp.	DDBJ/EMBL/ Gen bank accession number is AJ 784992.	Increases the virulence against <i>Fusarium</i> spp.	Gallo et al.	2004
8	<i>T.virens</i> strain IMI 304061 (<i>TgaA</i> , <i>TgaB</i> genes)	Antagonism against <i>S. rolfsii</i> and <i>R. solani</i> .	GenBank AY186729 (<i>tgaA</i>) and AY168002 (<i>tgaB</i>).	Increases virulence in the plant pathogenic interactions.	Mukherjee et al.	2004
9	<i>T.virens</i> wildtype strain Gv29-8 and an arginine auxotrophic strain, Tv10.4 (<i>tvsp1</i> serine protease encoding gene)	Involved in pathogenesis or biocontrol process of <i>R. solani</i> .	GenBank AY242844	Exhibits moderate activity against <i>R. solani</i> .	Pozo et al.	2004
10	<i>T.hamatum</i> strain LU593 (chitinase <i>chit42</i> and proteinase <i>prb1</i> gene)	Mycoparasitic activity against <i>Sclerotinia sclerotiorum</i> .	GenBank ITS1-AY241456, <i>chit42</i> -AY258898, <i>prb1</i> -AY258899, <i>xbg1.3</i> -110-AY269826	Exhibits moderate biocontrol activity.	Steyaert et al.	2004

Table 1. Continue.

11	<i>T. viride</i> IFO31137 (endo- β -1-6-galactanase gene)	A type of arabinogalactan proteins that involves in cell-cell adhesion, expansion and cell death.	Accession number not available	Expression of gene enhances the production of proteins.	Kotake et al.	2004
12	<i>T. atroviride</i> strain P1ATCC 74058 (<i>tga1</i> gene)	Chitinase formation and production of antifungal metabolites.	GenBank AY190117	Increases the antifungal activity.	Reithner et al.	2005
13	<i>T. virens</i> IMI 304061 (<i>tmkA</i> gene)	Induction of plant systemic resistance and biocontrol activity against <i>R. solani</i> . (Tested in green house condition)	Accession number not available	Shows increased biocontrol activity.	Viterbo et al.	2005
14	<i>T. harzianum</i> CECT 2413 (<i>ThPTR2</i> gene)	Di/tri peptide transporter gene, that involves in mycoparasitic activity against <i>B. cinerea</i> .	GenBank AJ868360	Induces peptide transport that enhances mycoparasitism	Vizcaino et al.	2006
15	<i>T. harzianum</i> CECT 2413 (<i>erg1</i> gene)	Silencing of the <i>erg1</i> gene enhances resistance to terbinafine that shows antifungal activity.	GenBank AM050097	Shows enhanced biocontrol activity.	Cardoza et al.	2006
16	<i>T. harzianum</i> Rifai CECT 2413 (<i>qid74</i> gene)	Involved in cell protection and adherence to hydrophobic surfaces that helps in antagonism against <i>R. solani</i> .	Accession number not available	Shows moderate biocontrol activity.	Rosado et al.	2007
17	<i>T. virens</i> Gv29-8 (<i>TvBgn2</i> and <i>TvBgn3</i> genes)	These genes help in encoding cell wall degrading enzymes.	Accession number not available	Shows enhanced biocontrol activity.	Dzonovic et al.	2007
18	<i>T. virens</i> IMI 304061 (<i>tac1</i> , adenylate cyclase gene)	Mycoparasitism against <i>R. solani</i> , <i>S. rolfssii</i> , <i>Pythium</i> Spp. and production of secondary metabolism.	Accession number not available	Shows reduced biocontrol activity.	Mukherjee et al.	2007
19	<i>T. harzianum</i> T88 (beta tubulin gene)	Exhibits biocontrol activities like mycoparasitism, and antifungal activity.	GenBank EF027167	Shows moderate biocontrol activity.	Li et al.	2007
20	<i>T. hamatum</i> LU593 (monooxygenase gene)	Antagonist activity against <i>S. sclerotiorum</i> , <i>S. minor</i> and <i>S. cepivorum</i> .	GenBank EU124654	Shows enhanced biocontrol activity.	Carpenter et al.	2008
21	<i>T. harzianum</i> E58 (<i>CRE1</i> gene)	Production of cellulase and hemicellulase enzymes.	Accession number not available	Shows enhanced biocontrol activity.	Saadia et al.	2008
22	<i>T. harzianum</i> CECT 2413 (T34 <i>hsp70</i>)	Increases fungal resistance to heat and abiotic stresses.	GenBank EU311400	Shows increased biocontrol activity.	MonteroBarrientos et al.	2008

Table 1. Continue.

23	<i>T.harzianum</i> (Th-Chit gene)	Antifungal activity in transgenic tobacco.	GenBank AJ605116	Shows activity.	enhanced	biocontrol	Saiprasad et al.	2009
24	<i>T.atroviride</i> P1 (ATCC 74058) (<i>Taabc2</i> gene)	Plays key role in antagonism against <i>R.solani</i> , <i>P.ultimum</i> , and <i>B.cinerea</i> .	Accession number not available	Shows activity.	decreased	biocontrol	Ruocco et al.	2009
25	<i>T.reesei</i> QM9414 (ATCC 26921) (<i>TrCCD1</i> gene)	Helps in hyphal growth, conidiospore development and carotenoid pigment production.	GenBank EF407410	Shows activity.	increased	biocontrol	HuaZhong et al.	2009
26	<i>T.harzianum</i> CECT 2413 (<i>Thctf1</i> transcription factor gene)	Antifungal activity against <i>R.solani</i> , <i>Fussarium oxysporum</i> and <i>B.cinerea</i> and production of 6-pentyl-2H-pyran-2.	GenBank EU551672	Shows activity.	enhanced	biocontrol	Rubio et al.	2009
27	<i>T.harzianum</i> (serine protease gene SL41)	Biocontrol activity against pathogens.	GenBank DQ910533	Shows activity.	enhanced	biocontrol	Liu et al.	2009
28	<i>T.harzianum</i> T34 CECT 2413 (endopolygalacturonase ThPG1 gene)	Secretion of plant cell wall degrading enzymes against <i>R. solani</i> and <i>P. ultimum</i> .	GenBank AM421521	Shows activity.	enhanced	biocontrol	MoranDiez et al.	2009
29	<i>T.asperillum</i> (Enzymology Group collection, UFG-ICB) (<i>tag 3</i> gene)	Production of cell wall degrading enzyme glucanase.	Accession number not available	Shows activity.	significant	biocontrol	Marcello et al.	2010
30	<i>T.virens</i> strain TvSMOE38 (<i>Sm1</i> gene, cysteine-rich protein)	A small cysteine-rich protein that induces defense responses in dicot and monocot plants and in protecting crop diseases.	Accession number not available	Shows activity.	enhanced	biocontrol	Buensanteai et al.	2010
31	<i>T.virens</i> (strain IMI 304061) (<i>TvGST</i> glutathione transferase gene)	Enhances cadmium tolerance in plants.	GenBank EH628505	Increases the biocontrol activity.			Dixit et al.	2011
32	<i>T.brevicompactum</i> IBT40841 (<i>tri5</i> gene)	Production of trichodermin and antifungal activity against <i>C. albicans</i> , <i>C. glabrata</i> and <i>A. fumigatus</i> .	Accession number not available	Shows activity.	enhanced	biocontrol	Tijerino et al.,	2011
33.	<i>T.harzianum</i> CECT 2413 (<i>Thke11</i> gene)	Expression of this gene in <i>A.thaliana</i> modulates glucosidase activity, and enhances tolerance to salt and osmotic stresses.	GenBank EU399786	Enhances the biocontrol activity.			Hermosa et al.	2011
34.	<i>T.harzianum</i> CECT 2413 (genes encoding for NAGases (<i>exc1</i> and <i>exc2</i>), chitinases (<i>chit42</i> and <i>chit33</i>), proteases (<i>prb1</i>) and b-glucanases (<i>bgn13.1</i>))	Mycoparasitic activity against <i>F. oxysporum</i> .	Accession number not available	Shows activity.	enhanced	biocontrol	Mondezar et al.	2011

from *T. harzianum*. The gene involves in the production of 6-pentyl-2H-pyran-2-one (6-PP) and shows antifungal activity against pathogens such as *R. solani*, *B. cinerea*, and *S. rolsii*. The sequences were analyzed using Lasergene package and cloned using pGEM-T vector (Rubio et al., 2009).

T. brevicompactum encodes, *tri5* a trichodiene synthase gene. The over expression of this gene helps in the production of trichodermin which shows antifungal activity against *S. cerevisiae*, *Kluyveromyces marxianus*, *Candida albicans*, *C. glabrata*, *C. tropicalis* and *Aspergillus fumigatus*. The sequences were analyzed using DNASTar package and aligned using CLUSTAL-X algorithm for analyzing the phylogenetic relationship. The gene *tri5* was cloned in pURSPT5 and transformed into *T. brevicompactum* (Tijerino et al., 2011).

From *T. harzianum*, endochitinase gene named *Th-Chit* was isolated, characterized and that gene confers antifungal activity in transgenic tobacco plant. Chitinase are one of the cell wall degrading proteins that help in the antifungal activity. The gene, *Th-Chit* was cloned using pTZ57R vector, and sequencing of the cloned cDNA was done by ABI prism automated DNA sequencer. From this the full length chitinase gene was isolated and then it is cloned in a binary vector named pIIHR-Th-Chit. The gene was transferred to tobacco plant and their presence was analyzed by polymerase chain reaction amplification from the control and transformed plants. Thus, Th-Chit gene confers antifungal activity against *A. alternata* (Saiprasad et al., 2009).

The *erg1* gene from *T. harzianum* was cloned and characterized. This gene encodes an enzyme named squalene epoxidase, which helps in the synthesis of ergosterol and silencing of this gene provides resistance to terbinafine, an antifungal compound. The antifungal activity was checked with *Saccharomyces cerevisiae*. pSIL-E1 vector was used to clone the gene *erg1*. Sequencing was done by DNASTar package and aligned using CLUSTAL-W algorithm. This is the first terpene biosynthesis gene characterized from *Trichoderma* genus (Cardoza et al., 2006).

Tga1 gene, the G protein α subunit of *T. atroviride* involves in production of chitinase and antifungal metabolites. Chitinase are the proteins that are involved in degrading the cell walls of pathogenic fungus. The sequences were cloned in the pGEM-T vector and characterized. The antifungal activity was determined by dual culture technique by plating wild type and mutant *Δtga1* strain of *T. atroviride* against plant pathogens such as *R. solani*, *B. cinerea*, and *S. sclerotiorum*. The antifungal activity between the wild and mutant type strains were analyzed by altering the *tga1* gene (Reithner et al., 2005).

In *T. harzianum*, a gene, viz., *ech42* codes for endochitinase was studied. The gene was cloned in pAN7-1 vector. Disruption of this gene affects the biocontrol activity of the fungus. The antifungal activity was tested

against pathogens like *B. cinerea*, and *R. solani* with the wild type and disruptant strains (Woo et al., 1999). From *T. hamatum* monooxygenase gene was isolated and characterized. This gene helps in the antifungal activity against some pathogens like *S. sclerotiorum*, *Sclerotinia minor*, and *Sclerotium cepivorum*. The expression of monooxygenase gene was influenced by until it had made contact with the two fungal species, and the expression seems to be more particularly at pH 4.

The DNA was isolated from *T. hamatum*, genomic library was constructed and it was sequenced. Agrobacterium mediated gene transformation was done with the help of pG3K02 and the gene was expressed. The promoter region of the monooxygenase gene was analyzed for the presence of motifs which helps in the regular expression of the genes. Thus, *T. hamatum* monooxygenase gene plays it significant role in the antagonist activity (Carpenter et al., 2008).

Hyphal growth

A new gene, *TrCCD1* from *Trichoderma reesei* was isolated and characterized. This gene involves in carotenoid metabolism that helps in the development of conidiospores and hyphal growth in *T. reesei*. The function of the gene was analyzed by comparing two mutant types named *ccdO* and *ccdP* (carotenoid cleavage dioxygenase) with the parental type. The T-DNA insertion of fungal genome was sequenced using specific primer, multiple sequence alignment was done using CLUSTAL-W algorithm and phylogenetic relationship was done by neighbor joining method (Zhong et al., 2009).

CONCLUSION

The various types of *Trichoderma* species were involved in the biocontrol activity and their mechanism of action were well known by the characterization and expression of the genes present in them. The fungal pathogens were known to cause major diseases in the agricultural scenario. So, most of the farmers were using hazardous chemical pesticides which cause major problems in the yield, ultimately affecting the land, soil fertility and remains toxic when consumed by humans and animals.

By using various microbial biocontrol agents, this problem has been reduced. The genes isolated from these biocontrol agents has been found to play an essential role in biocontrol activity therefore, with the help of genetic engineering techniques still more number of beneficial genes should be discovered in developing the agriculture for our future generations.

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