Trichoderma and biocontrol genes: Review

Shahzad Munir¹, Qaiser Jamal¹, Kalsoom Bano², Sikandar Khan Sherwani³, Muhammad Naseer Abbas¹, Sikandar Azam⁴, Abdullah Khan¹, Asadullah¹, Sardar Ali¹, Muhammad Anees¹

1. Kohat University of science and technology, Kohat, Pakistan
2. Quaid-i-Azam University Islamabad, Pakistan
3. Federal Urdu University of Arts, Science and Technology Karachi, Pakistan
4. National University of Science and Technology Islamabad, Pakistan
Corresponding author email: shazid_10@yahoo.com

ABSTRACT

Trichoderma spp. are known worldwide for their antagonistic activities against different phytopathogens. Fungal genus Trichoderma plays a major role in biocontrol of the plant diseases. Different kinds of biocontrol enzymes are produced by these Trichoderma which have a significant role in cell wall degradation, hyphal growth and antagonistic activity against phytopathogens. Different genes are responsible for this biocontrol activity which has a role in antagonism. We can easily study the function and role of these genes in biocontrol mechanism by the advance molecular biological techniques. The genes which are responsible for biocontrol activity can be isolated, characterized, clone, sequence and further the functions of the genes can be expressed. The present review is based on the biocontrol genes of Trichoderma longibrachiatum, Trichoderma harzianum, Trichoderma hamatum, Trichoderma reesei, Trichoderma atroviride and Trichoderma viride.

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Introduction

Many plant pathogens that cause major diseases in agricultural fields are control by the well-known biocontrol agents Trichoderma. Trichoderma species are recognized for their production of enzymes called cell wall degrading enzymes (CWDEs) that can be used for industrial production. All living organisms are made up of genes that code for a protein which performs the particular functions. Some genes that play an important role in the biocontrol process are known as the biocontrol genes. These genes send some signals which help in secretion of proteins and enzymes that degrade the plant pathogens. The plant growth has been promoted and prevented from phytopathogen attack if the expressions of these genes are increased results in enhanced biocontrol activity. Consequently the biocontrol genes can be produced and cloned in huge quantities for commercial uses (Massart and Jijakli, 2007).

Resistance to the biotic and abiotic stresses such as heat, drought and salt can be provided by used of some genes of Trichoderma species (Kuc, 2001). The biocontrol mechanism of a specific species should be well recognized before using as a commercial product (Grondona et al., 1997). Antibiosis, mycoparasitism and providing plant nutrition are the major biocontrol process (Janisiewicz and Korsten, 2002).

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

Roles Of Biocontrol Genes

The genus Trichoderma acts as a biocontrol agent due to its feasible character in fighting against the pathogens. Chitinase, tubulins, protease, xylanase, galacturonase, glucanase, stress tolerant genes and cell adhesion proteins are the major kind of biocontrol genes that can be easily isolated, cloned and characterized. Cell wall degradation, hyphal growth, parasitic activity and stress tolerance are the major biocontrol mechanism by these genes. Microtubules formed the structural proteins tubulins which help in studying the composition of cell wall of pathogens (Li et al., 2010). Glycosidic bonds are
breakdown by chitinase enzymes. D-glucose to D-glucono-1, 5-lactone and hydrogen peroxide catalyses by antifungal enzyme glucose oxidase (Ciliénto et al., 2004). Hemicellulose can be break by xylanase which is major component of plant cell walls.

Use Of Biocontrol Genes
Degradation of fungal cell wall

Tvsp1 is a gene which was cloned successfully from Trichoderma virens and its function was analyzed and it encode for serine protease. Rhizoctonia solani which affects the cotton seedlings has been controlled biologically by serine protease. In Escherichia coli the gene tvsp1 was expressed and pET-30 vector was used for their cloning. Thus, the fungal cell wall is easily degraded by serine protease (Pozo et al., 2004).

In T. harzianum, trichodiene synthase gene tri5 was isolated and characterized. This tri5 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 tri5 was isolated by designing of specific primers. The sequence was inserted into pGEM-T vector, cloned and expressed.

The presence of tri5 gene was confirmed by screening with other Trichoderma isolates (Gallo et al., 2004). 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. 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) (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 Rhiizopus oryzae (Djionovic et al., 2007).

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 (Table 1). 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 (Buensantea et al., 2010).

From Trichoderma atroviride a gene, gluc78 which codes for an antifungal glucan 1, 3-β-glucosidase was isolated, cloned and sequenced. 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).

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 DH5α. Thus, serine proteases were cloned and characterized (Liu et al., 2009).

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. virens, 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)

The gene, TvPG1 was isolated from T. harzianum and characterized which encodes for endopoly-galacturonase. 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 T. virens TgP1 gene was obtained by polymerase chain reaction and was cloned in pSlIpG1 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→6)-galactanase gene was isolated from T. viride, cloned and expressed in E. coli. Galactanase enzymes belong to the family of arabinoxylactan proteins that involve in cell-cell adhesion, cell expansion and cell death. The cDNA clone of the gene Tv6Gal was done by RT-PCR, cloned in pGEM-T vector and expressed in E. coli (Kotake et al., 2004).
Antifungal activity

Endochitinase gene named Th-Chit was isolated, characterized from T. harzianum 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).

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, Kluveromyces marxianus, Candida albicans, C. glabrata, C. tropicalis and Aspergillus fumigatus. The sequences were analyzed using DNA star 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).

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 terbinafen, 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 DNA star package and aligned using CLUSTAL-W algorithm. This is the first terpene biosynthesis gene characterized from Trichoderma genus (Cardoza et al., 2006).

A transcription factor gene named Thetf1 was isolated 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. rolfsii. The sequences were analyzed using Laser gene package and cloned using pGEM-T vector (Rubio et al., 2009). Tgal 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 Δtgal 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 tgal gene (Reithner et al., 2005).

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. Agro- bacterium 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). In T. harzianum, a gene, viz., ech42 codes for endo- chitinase 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 disruptive strains (Woo et al., 1999).

Stress tolerances: Biotic and abiotic

Trichoderma species helps the plant to survive in the abiotic stress conditions. 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). From T. harzianum T34 isolate, hsp70 gene was cloned in pGEM-T vector and expressed in different isolates of T. harzianum and characterized which 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 DNA star package and aligned using CLUSTAL X algorithm. (Manterobarrientos et al., 2008).

A gene named Thkel1 was isolated and characterized from the fungus 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. 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).

Mycoparasitic action

Genes were cloned and expressed from five isolates of T. harzianum namely (T 30, 31, 32, 57 and 78) encoding for N-acetyl-β-D-glucosaminidase (exc1 and exc2), chitinase (chit42 and chit33), protease (prb1) and β-glucanase (bgn 13,1). 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 (Mondejar et al., 2011).
Table 1. Biocontrol Genes and their functions

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Biocontrol Gene</th>
<th>Name of Isolate</th>
<th>Effect of Gene in Biocontrol</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tga1 gene</td>
<td>T.atroviride strain P1 ATCC 74058</td>
<td>Increases the antifungal Activity</td>
<td>Reithner et al. 2005</td>
</tr>
<tr>
<td>2</td>
<td>TmkA gene</td>
<td>T. virens IMI 304061</td>
<td>Shows increased biocontrol activity</td>
<td>Viterbo et al. 2005</td>
</tr>
<tr>
<td>3</td>
<td>ThPTR2 gene</td>
<td>T. harzianum CECT 2413</td>
<td>Induces peptide transport that enhances mycoparas-istism</td>
<td>Vizcaino et al. 2006</td>
</tr>
<tr>
<td>4</td>
<td>Erg1 gene</td>
<td>T. harzianum CECT 2413</td>
<td>Shows enhanced biocontrol activity</td>
<td>Cardoza et al. 2006</td>
</tr>
<tr>
<td>5</td>
<td>Qid74 gene</td>
<td>T. harzianum Rifaï CECT 2413</td>
<td>Shows moderate biocontrol Activity</td>
<td>Rosado et al. 2007</td>
</tr>
<tr>
<td>6</td>
<td>T34 hsp70</td>
<td>T. harzianum CECT 2413</td>
<td>Shows enhanced biocontrol activity</td>
<td>Dzonovic et al. 2007</td>
</tr>
<tr>
<td>7</td>
<td>Beta tubulin</td>
<td>T. harzianum T88</td>
<td>Shows moderate biocontrol Activity</td>
<td>Li et al. 2007</td>
</tr>
<tr>
<td>8</td>
<td>Monoxygenase</td>
<td>T. hamatum LU593</td>
<td>Shows enhanced biocontrol activity</td>
<td>Carpenter et al. 2008</td>
</tr>
<tr>
<td>9</td>
<td>TrCCD1 gene</td>
<td>T. reesei QM9414 (ATCC 26921)</td>
<td>Shows increased biocontrol activity</td>
<td>HuaZhong et al. 2009</td>
</tr>
<tr>
<td>10</td>
<td>Serine protease SLA1</td>
<td>T. harzianum</td>
<td>Shows enhanced biocontrol activity</td>
<td>Liu et al. 2009</td>
</tr>
<tr>
<td>11</td>
<td>Sm1 gene</td>
<td>T. virens strain TvSMOE38</td>
<td>Shows enhanced biocontrol Activity</td>
<td>Buensantea et al. 2010</td>
</tr>
<tr>
<td>12</td>
<td>Tag 3 gene</td>
<td>T. asperellum</td>
<td>Shows significant biocontrol Activity</td>
<td>Marcello et al. 2010</td>
</tr>
<tr>
<td>13</td>
<td>Thke11 gene</td>
<td>T. harzianum CECT 2413</td>
<td>Enhances the biocontrol activity</td>
<td>Hermosa et al. 2011</td>
</tr>
<tr>
<td>14</td>
<td>Tri5 gene</td>
<td>T. brevicompactum IBT40841</td>
<td>Shows enhanced biocontrol Activity</td>
<td>Tijerino et al., 2011</td>
</tr>
</tbody>
</table>

*T. longibrachiatum* transformants showed over expression of β-1,4-endoglucanase gene *eggl*. This gene showed biocontrol activity against *P. ultimum* in damping-off of cucumber. The *eggl* gene, coding for endoglucanase was isolated from *T. longibrachiataum*, 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 *eggl* gene showed good biocontrol activity (Migheli et al., 1998). TmkA, mitogen activated protein kinase from *T. Virens* is known to cause myco-parasitic activity against *R. solani* and *S. rolsii* (Mukherjee et al., 2003).

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 *prbl* and endochitinase *ech42* were isolated and characterized. These genes involved in the mycoparasitic activity against *R. solani* and *S. rolsii*. 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 *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 ThpPTR2 gene was obtained through reverse transcript polymerase chain reaction, transferred into pIBRC43 plasmid, cloned and expressed. The sequences were aligned using CLUSTAL-W algorithm and protein binding motifs were discovered. The mycoparasitic expression of the ThpPTR2 gene was analyzed by dual culture assay (Vizcaino et al., 2006).

In *Trichoderma hamatum* the expression of mycoparasitism genes, namely chinatine *chit*42 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).

**Genes responsible for 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 dioxy- genase) 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 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. *Trichoderma* species were involved in the biocontrol activity and their mechanisms of action were well known by the characterization and expression of the genes present in them. This problem has been reduced by use of microbial biocontrol genes. With the help of genetic engineering techniques still more number of beneficial genes should be discovered in developing the agriculture and the genes isolated from these biocontrol agents has been found to play an essential role in biocontrol activity.

**References**


