

Review

Biocontrol mechanisms by *Trichoderma* through genomics and proteomics analysis: A review

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Soil-borne phytopathogenic fungi pose serious threats to yield of several crops. Biological control is an eco-friendly approach in the effective management of crop diseases. *Trichoderma* is an important soil-borne fungus, which play an important role in antagonism by secretion of different hydrolytic enzymes. Lentil is an important pulse crop world-wide and its yield is severely affected by wilt disease. Plant pathological and molecular dissemination of the genus *Trichoderma* have reached the ultimate level of research and have been figured in almost all the pioneers of scientific publications. Enough of this exploration and exploitation! Now it is time to discover the *in silico* approach of this fungi. Many features and roles of this fungus are of biological importance that cannot be left unseen such as biological control mechanism, plant growth promoter, dying agent, etc. to name a few. Bioinformatics methods, tools and protocols, when applied to the genome of *Trichoderma* species, have provided many valuable and considerable results. Genomics tools and methods brought new genes that play important roles in mycoparasitism and biocontrol mechanisms for plant protection against pathogens. Open reading frames, promoter regions, gene-finding, primer designing, domain analysis, motif prediction, similarity searching in BLASTn, BLASTp, BLASTx have predicted some important regions of interest that have been found to be functional in the genome.

Key words: Genomics, *Trichoderma*, Mycoparasitism, cell wall degrading enzymes (CWDEs), bioagent.

INTRODUCTION

Many plant pathogens that cause major diseases in agricultural fields are controlled by the well-known biocontrol agent, *Trichoderma*. *Trichoderma* species are recognized for their production of enzymes called cell wall degrading enzymes (CWDEs) such as xylanase and glucanase (Pandey et al., 2014) that can be used for

industrial production. All living organisms are made up of genes that code for a protein which performs the particular function. 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

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pathogens. These biocontrol genes can be cloned in huge quantities and can be used on large scale for commercial production (Massart and Jijakli, 2007).

Some *Trichoderma* genes are also helpful in providing resistance to the biotic and abiotic stresses such as heat, drought and salt (Kuc, 2001). The major biocontrol processes include antibiosis, mycoparasitism and providing plant nutrition (Janisiewicz and Korsten, 2002).

Trichoderma harzianum is the most effective strain among the various species of the *Trichoderma* used for biocontrol mechanisms (Gao et al., 2002).

The fungal pathogens were known to cause major diseases in the crops. 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 their genes. This problem has been reduced by use of microbial biocontrol genes. With the help of genetic engineering techniques, more beneficial genes should still be discovered to develop the agriculture. Genes isolated from these biocontrol agents have been found to play an essential role in biocontrol activity. Chitinase, tubulins, protease, xylanase, galacturonase, glucanase, stress tolerant genes and cell adhesion proteins are the major kind of biocontrol genes (Pandey et al., 2014) 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.

ROLES OF BIOCONTROL GENES

The sequencing of fungal genomes is advancing at breakneck-speed, producing voluminous amounts of data. Within the next five years, it is possible that over a couple thousand genomes, representing every major fungal family will be completed and available to the scientific community. In order for this data to have a truly transformative effect on mycological and other research, several factors need to be addressed. These include: (1) the establishment of user friendly platforms for examining, sorting, and sifting through the genomes, (2) integration, or at least cross-communication, between the various databases that house the genomic data, and (3) investment in community resources that can act as repositories and provide materials to researchers, that is, strains, clones, plasmids, etc. The frameworks for some these needs, e.g. the materials available from the Fungal Genetics Stock Center (FGSC, University of Missouri), are already established and should be reinforced, whereas for others, e.g. data accessibility, the sooner a plan can be implemented, the better. The Fungal Kingdom is considered to contribute greater than 15% of the species richness found in the major groups of organisms. This study is a reflection of the usefulness of sequence

analysis of the 28S ribosomal RNA gene in identifying fungal as well as determining fungal diversity. Various techniques that are based on utilizing the 28S rRNA have been discussed. Of critical importance is the manner in which massively parallel sequencing was exploited to correct the under representation of fungal species in compilations of fungal that were drawn using traditional methods of surveying fungal species from ecosystems (Srivastava et al., 2014).

GENES INVOLVED IN BIOCONTROL MECHANISMS

Degradation of fungal cell wall

The gene *Tvsp1* was cloned successfully from *Trichoderma virens*, its function was analyzed and it was proved to encode for serine protease. *Rhizoctonia solani* which affects the cotton seedlings has been controlled biologically by serine protease (Poza et al., 2004).

The objectives of this research were to characterize isolates of *Trichoderma* collected from rhizospheres of chickpea, pigeonpea and lentil crop from different places of Uttar Pradesh, India, using microsatellite-primed polymerase chain reaction (MP-PCR) and ribosomal DNA (rDNA) sequence analysis and to combine these results with morphological characteristics for classification.

Thirty isolates of *T. viride* obtained from rhizosphere soil of plantation crops, and agricultural fields of UP region were studied using ISSR and ITS-PCR. The genetic relatedness among thirty isolates of *T. viride* was analyzed with six microsatellite primers. ISSR profiles showed genetic diversity among the isolates with the formation of eight clusters. Analysis of dendrogram revealed that similarity coefficient ranged from 0.27 to 0.95. ITS-PCR of rDNA region with ITS1 and ITS4 primers produced 600 bp products in all isolates. This result indicated the identification patterns of *Trichoderma* isolates (Shahid et al., (2014).

In *T. harzianum*, trichodiene syntheses gene *tri5* was isolated and characterized. This *tri5* gene is 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 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 reac-

tion (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 *Rhizopus oryzae* (Djonovic 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*. 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 -tubulingene 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). 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 pathogen's cell wall degradation. 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 involved in the pathogen's cell wall degradation. 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* DH5- α . 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 carbon source. The full length DNA 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 *ThPG1* was isolated from *T. harzianum*, characterized and proved to encode for endopolygalacturonase.

This enzyme is involved in the cell wall degradation of pathogens like *R. solani* and *P. ultimum*. 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 (Moran Diez 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 involved 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 from *T. harzianum* that is responsible for the antifungal activity in transgenic tobacco plant. The gene *tri5* from *T. brevicompactum* encodes a trichodiene syntheses. The over expression of this gene helps in the production of trichodermin which shows antifungal activity against *S. cerevisiae*, *Kluyveromyces marxianus*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Aspergillus fumigates*.

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 *S. cerevisiae*. pSIL-E1 vector was used to clone the gene *erg1*. This is the first terpene biosynthesis gene characterized from *Trichoderma* genus (Cardoza et al., 2006). From *T. hamatum*, monooxygenase gene was isolated and characterized. This gene helps in the antifungal activity against some pathogens like *Sclerotinia sclerotiorum*, *Sclerotinia minor* and *Sclerotium cepivorum*. The expression of monooxygenase gene was influenced by the two fungal species contact, and its expression seems to be particularly important at pH 4.

The morphological, physiological, molecular characterization and bio-formulation of *T. harzianum* (Th Azad) and *T. viride* 01PP-8315, an effective fungicide and a biological control agent that protects the plants and seeds from other pathogenic fungi, have been investigated. The physiologic study is done in an attempt to find the effective management of the disease caused by soil borne pathogens. *T. harzianum* (Th Azad) and *T. viride* 01PP-8315 were isolated from the infected field's soil samples of pigeon pea grown at preferable temperatures,

pH and from different solid and liquid culture media. Most preferable temperature for the growth and sporulation of *T. harzianum* (Th Azad) and *T. viride* 01PP-8315 has been observed to be up to 30°C (210.5 mg dry weight of mycelium). A detailed morphology of the strain is described in this study including colony growth rate, colony color, colony edge, mycelial form, conidiation, conidiophore branching, conidial wall, conidial color, etc. The molecular characterization of the strain is carried out using an 18S rRNA gene sequence with the help of a universal Internal Transcribed Spacer marker that gives an amplicon of a total of 1173 base pairs. The 546 pb 18S rRNA gene was sequenced and used for the identification of isolated fungal strains that is later sequenced and allotted with Gene Bank Accession no. JX119211 and KC800922, respectively. To check the presence of endochitinase gene in two of the potential strains of *Trichoderma* species viz. *T. harzianum* (Th Azad) and *T. viride*, 01PP an ech42 primer was used. A talc based bioformulation has been prepared with this strain where the population of the spores was found to decline after 180 days (Shahid et al., 2014).

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, expressed in different isolates of *T. harzianum* and characterized as a gene that helps in increasing the fungal resistance to heat and salt tolerance, osmotic and oxidative tolerances. The protein sequences were analyzed using DNA star package and aligned using CLUSTAL X algorithm (Mantero Barrientos et al., 2008).

A gene named *Thkel1* was isolated and characterized from the fungus *T. harzianum*. This gene codes for putativekelch-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.

Trichoderma longibrachiatum transformants showed over expression of β-1,4-endoglucanase gene *egl1*. This gene showed biocontrol activity against *P. ultimum* in cucumber's damping-off. The *egl1* gene, coding for endoglucanase was isolated from *T. longibrachiatum*, cloned and expressed in *S. 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).

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.

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 gene was cloned using pGEM-T vector and their expression was analyzed using RT-PCR. The antagonist activity against pathogens such as *R. solani*, *Botrytis cinerea*, and *P. ultimum* was done by dual culture plate assay with *T. atroviride* wild and mutant type strains (Ruocco et al., 2009).

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 *B. cinerea*.

Genes responsible for hyphal growth

A new gene, named *TrCCD1* was isolated from *Trichoderma reesei*. This gene is involved 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).

BIOINFORMATIC APPROACHES

Proteomic techniques are able to separate and characterize complex sets of proteins. Moreover, the vast majority of current drug targets are proteins. As well as using proteomics to settle drug targets of a specific compound or to search new therapeutics objectives, the development of mathematical algorithms to predict its role may be a useful tool for drug discovery through proteomics analysis. Xu et al. (2007) presented the concept of “drug target-likeness” of a protein as an independent set of characteristics of successful targets. By a thorough study of known drug targets, it is possible to determine if an obtained protein sequence fits with this drug target role. This methodology may open a new frontier in fungicide design, algorithm applies to fungal plant pathogens, accumulation of molecular information on these organisms, and commercial interest of developing environmentally friendly fungicides, will press the research community to improve mathematical algorithms to predict the role of a protein as a fungicide. Proteomics methods helped in finding the functional peptides that show significant functions in the biocontrol mechanism. These peptides have successfully been identified in the peptide mass fingerprinting analysis through MALDI-TOF mass spectrometry. *T. viride* and *T. harzianum* consist of numerous peptides that are clearly visible in the mass spectra. Cell wall degrading enzymes such as chitinase, glucanase, proteinase and xylanase have been identified in the *Trichoderma* species under consideration and protein structural determination of the modeled structures (in UCSF Chimera) bring in front the N- and C-termini and the active sites of the proteins.

New protein-based strategies to classical chemical fungicide design

Historically, drugs have been obtained from plant and animals products, from derivatives of human endogenous legends or from chemicals or semi-synthetic chemicals. Classical methods to control fungal plant diseases are based on the use of chemical compounds. In spite of the success achieved, new criteria for the indiscriminate use of toxic compounds in nature avoid using this technology. Control strategies based on classical fungicides produce serious collateral effects, mainly related with environmental pollution and the development of multidrug resistance.

Several changes in the design of chemical fungicides are being tackled by the research community by summarizing the genomic and proteomic information available. Biosynthetic fungicide design has been established as a new focus in fungicide development (Collado and Sanchez, 2007). Based on a deep study of fungal biology, the use of alien or modified natural compounds provides a potential species-specific method of controlling plant pathogens by specific inhibition of those proteins involved in the infection cycle (Pinedo et al., 2008).

The use of these compounds minimizes the environmental impact as they are biodegradable, possess high specificity, and integrate poorly in the food chain.

In the post-genomic era, new terms related with chemical “-omics” have appeared. The term “genetic chemical” describes the use of small molecules that selectively perturb gene function. When this concept is applied on a genome-wide scale it is named “chemogenomics”. The application of chemogenomics to protein targets is named “chemoproteomics”; although a more explicit definition is targeted related affinity profiling (TRAP) defined as the use of biology to inform chemistry (Beroza, 2002). The accumulation of proteomic information of fungal plant pathogens may be an incentive to the development of new and environmentally friendly fungicides.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

- Beroza P, Villar HO, Wick MM, Martin GR (2002). Chemoproteomics as a basis for post-genomic drug discovery. *Drug Discov. Today* 7:807–814.
- Buensanteai N, Mukherjee PM, Horwitz BA, Cheng C (2010). Expression and purification of biologically active *Trichoderma virens* proteinaceous elicitor Sm1 in *Pichia pastoris*. *Protein Express Purif.* 72: 131-138.
- Cardoza RE, Vizcaino JA, Hermosa MR, Gonzalez FJ, Llobell A, Monte M, Gutierrez S (2006). Cloning and characterization of the *erg1* gene of *Trichoderma harzianum*: Effect of the *erg1* silencing on ergosterol biosynthesis and resistance to terbinafine. *Fungal Genet. Biol.* 43: 164-178.
- Collado IG, Sanchez AJMA, Hanson JR (2007). Fungal terpene metabolites: Biosynthetic relationships and the control of the phytopathogenic fungus *Botrytis cinerea*. *Nat. Prod. Rep.* 24: 674–686.
- Dixit P, Mukherjee PK, Ramachandran V, Eapen S (2011). Glutathione transferase from *Trichoderma virens* enhances cadmium tolerance without enhancing its accumulation in transgenic *Nicotiana glauca*. *PLoS ONE* 6(1): 1-15.
- Djonovic S, Vittone G, MendozaHerrera A, Kenerley CM (2007). Enhanced biocontrol activity of *Trichoderma virens* transformants constitutively coexpressing β -1,3- and β -1,6-glucanase genes. *Mol. Plant Pathol.* 8(4): 469-480.
- Donzelli BGG, Lorito M, Scala F, Harman GE (2001). Cloning, sequence and structure of a gene encoding an antifungal glucan 1,3 β -glucosidase from *Trichoderma atroviride* (*T. harzianum*). *Gene* 277: 199-208.

- Gallo A, Mule G, Favilla M, Altomare C (2004). Isolation and characterization of a trichodiene synthase homologous gene in *Trichoderma harzianum*. *Physiol. Mol. Plant Pathol.* 65: 11-20.
- Gao KX, Liu XG, Liu YH, Zhu TB, Wang SL (2002). Potential of *Trichoderma harzianum* and *T. atroviride* to control *Botrytis sphaeriaberengeriana* f. sp. *piricola*, the cause of apple ring rot. *J. Phytopathol.* 150: 271-276.
- Hermosa R, Botella L, Keck M, Jimenez JA, Montero Barrientos M, Arbona V, Gomez Cadenas A, Monte E, Nicolas C (2011). The over expression in *Arabidopsis thaliana* of a *Trichoderma harzianum* gene that modulates glucosidase activity, and enhances tolerance to salt and osmotic stresses. *J. Plant Physiol.* 168: 1295-1302.
- Kotake T, Kaneko S, Kubomoto A, Haque MA, Kobayashi H, Tsumuraya Y (2004). Molecular cloning and expression in *Escherichia coli* of a *Trichoderma viride* endo- β -(1 \rightarrow 6)-galactanase gene. *Biochem. J.* 377: 749-755.
- Kuc J (2001). Concepts and direction of induced systemic resistance in plants and its application. *Eur. J. Plant Pathol.* 107: 7-12.
- Li M, Yang Q (2007). Isolation and characterization of a β -tubulin gene from *Trichoderma harzianum*. *Biochem. Genet.* 45: 529-534.
- Liu Y, Yang Q, Song J (2009). A new serine protease gene from *Trichoderma harzianum* is expressed in *Saccharomyces cerevisiae*. *Appl. Biochem. Microbiol.* 45(1): 22-26.
- Mantero Barrientos M, Hermosa R, Nicolas C, Cardoza RE, Gutierrez S, Monte E (2008). Over expression of a *Trichoderma HSP70* gene increases fungal resistance to heat and other abiotic stresses. *Fungal Genet. Biol.* 45: 1506-1513.
- Marcello CM, Steindorff AS, Silva SP, Silva RN, Bataus LAM (2010). Expression analysis of the exo- β -1,3-glucanase from the mycoparasitic fungus *Trichoderma asperellum*. *Microbiol. Res.* 165: 75-81.
- Massart S, Jijakli HM (2007). Use of molecular techniques to elucidate the mechanisms of action of biocontrol agents: A review. *J. Microbiol. Methods* 69: 229-241.
- Migheli Q, Gonzalez Candelas L, Dealessi L, Camponogara A, Ramon Vidal D (1998). Trans formants of *Trichoderma longibrachiatum* overexpressing the β -1,4- endoglucanase gene *egl1* show enhanced biocontrol of *Pythium ultimum* on cucumber. *Biol. Control* 88 (7): 673-677.
- Min YS, Kim BG, Lee C, Ho Gil H, Joong Hoon AHN (2002). Purification, Characterization, and cDNA cloning of Xylanase from fungus *Trichoderma* strain SY. *J. Microbiol. Biotechnol.* 12(6): 1-5.
- Moran Diez E, Hermosa R, Ambrosino P, Cadoza RE, Gutierrez S (2009). The ThPG1 endopolygalacturonase is required for the *Trichoderma harzianum*-Plant beneficial interaction. *Am. Phytopathol. Soc.* 22(8): 1021-1031.
- Mukherjee PM, Latha J, Hadar R, Horwitz BA (2004). Role of two G protein alpha subunits, TgaA and TgaB, in the antagonism of plant pathogens by *Trichoderma virens*. *Appl. Environ. Microbiol.* 70(1): 542-549.
- Mukherjee M, Mukherjee PK, Kale PS (2007). cAMP signalling is involved in growth, germination, mycoparasitism and secondary metabolism in *Trichoderma virens*. *Microbiology* 153: 1734-1742.
- Pandey S, Shahid M, Srivastava M, Sharma A, Singh A, Kumar V, Srivastava Y (2014) Isolation and Optimized Production of Xylanase under Solid State Fermentation Condition from *Trichoderma* sp. *Int. J. Adv. Res.* 2 (3): 263-273.
- Pandey S, Shahid M, Srivastava M, Sharma, A, Singh A, Kumar V (2014). Isolation purification and characterization of glucanase enzyme from antagonistic fungus *Trichoderma*. *Int. J. Sci. Eng. Res.* 5(3): 646-649.
- Pinedo C, Wang CM, Pradier JM, Dalmais BRR, Choquer M, Le Pelcheur P, Morgant G, Collado IG, Cane DE, Viaud M (2008). Sesquiterpene synthase from the botrydial biosynthetic gene cluster of the phytopathogen *Botrytis cinerea*. *ACS Chem. Biol.* 3:791-801.
- Pozo MJ, JongMin B, Garcia JM, Kenerley CM (2004). Functional analysis of *tvsp1*, a serine protease-encoding gene in the biocontrol agent *Trichoderma virens*. *Fungal Genet. Biol.* 41: 336-348.
- Ruocco M, Lanzuise S, Vinalé F, Marra R, Turra D, LoisWoo S, Lorito M (2009). Identification of a New Biocontrol Gene in *Trichoderma atroviride*: The Role of an ABC Transporter Membrane Pump in the interaction with different plant-pathogenic fungi. *Am. Phytopathol.*
- Saadia M, Ahmed S, Jamil A (2008). Isolation and cloning of *cre1* gene from a filamentous fungus *Trichoderma harzianum*. *Pak. J. Bot.* 40(1): 421-426.
- Shahid M, Singh A, Srivastava M, Srivastava DK (2014). Molecular Characterization of *Trichoderma viride* isolated from Rhizospheric Soils of Uttar Pradesh Based on rDNA Markers and Analysis of Their PCR-ISSR Profiles. *J. Mol. Biomark. Diagn.* 5(2): 1000169.
- Shahid M, Srivastava M, Kumar V, Singh A, Pandey S (2014). Genetic determination of potential *Trichoderma* species using ISSR (Microsatellite) marker in Uttar Pradesh. *J. Microb. Biochem. Technol.* 6(3): 174-178.
- Shahid M, Srivastava M, Singh A, Kumar V, Pandey S (2014). Comparative study of biological agents, *Trichoderma harzianum* (Th-Azad) and *Trichoderma viride* (01PP) for controlling wilt disease in pigeon pea. *J. Microb. Biochem. Technol.* 6(2): 110-115.
- Srivastava M, Shahid M, Singh A, Kumar V (2014). To develop strain specific molecular marker for easy and proper identification of fungal species based on molecular characters: A Review. *J. Mol. Biomark. Diagn.* 5(3): 1000172.
- Xu H, Lin M, Wang W, Li Z, Huang J, Chen Y, Chen X (2007). Learning the drug target-likeness of a protein. *Proteomics* 7(23):4255-4263.
- Zhong YH, Wang TH, Wang XL, Zhang GT, Yu HN (2009). Identification and characterization of a novel gene, TrCCD1, and its possible function in hyphal growth and conidiospore development of *Fungal Genet. Biol.* 46: 255-263.