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Advances in Bioresearch

ORIGINAL ARTICLE

In-silico Characterization of Antagonistic Protein of *Trichoderma spp.* against Pathogenic Protein of *Fusarium oxysporum* f. sp. *lycopersici*

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ABSTRACT
Fusarium oxysporum f. sp. lycopersici (Fol) is a fungus that is a major problem for tomato plants, causing wilt. Trichoderma spp. is also a group of filamentous fungi used as a biocontrol agent reported to have antagonistic activity for important plant pathogens. For further research we used the protein domain of the pathogenic protein of Fol and the antagonistic protein of Trichoderma spp., and analyzed the Secondary structure, Protein-protein interaction (PPI) via bioinformatics tools. So far we have the antagonistic behavior of Trichoderma spp. against Fol. As a result, Fol contained conserve domains in pathogenic proteins such as AhpC-TSA, Redoxin domain and family, and other small domains were also involved, identified as ZnF_CHCC domain, Prim_Zn_Ribbon domain, PSA domain, Knot1 domain, WR1 domain, LRRCT domain, agouti domain, CXC domain, TRASH, ZnF_Rad18 domain and ACR domain, all these domains were significant for the survival of Fol in tomato plants as they suppress plant immunity and until after survive the death of the plant. In addition, Trichoderma spp. also contained different types of domains named PKS_AT, PKS_PP, A_NRPS_SidN3_like, Endochitinase, FUM14_C_NRPS-like, HWK_HK, Endochitinase 42, Proteasome_A_N, Antimicrobial21, PKS_ER and EntF, which was significant in inhibiting Fol or mycoparasitic behavior. Besides Trichoderma spp. an antagonistic expression for Fol was shown. All Trichoderma spp. contained domain in antagonistic protein to Fol, as endochitinase, secondary metabolites and antimicrobial domains are involved as inhibitor of Fol. Understood nature of protein domain isolated from the pathogenic behavior of Fol and the antagonistic behavior of Trichoderma spp. at the molecular level, this information will be useful in studies of interaction of Trichoderma spp. with Fol in the future.
***Keywords**: Protein domains, Biocontrol agents, Trichoderma spp. domains, Pathogenic protein domains of Fol.*

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INTRODUCTION

Fusarium oxysporum f.sp. Lycopersici (*Fol*) is a soil-dwelling fungus that causes wilting in tomato plants. Where, 25-55% incidence of wilt was recorded in tomato plant. However, 30-40% yield loss is also noted, which can reach up to 80% in India under favorable conditions. Three races, race1, race2 and race3, have been recorded in *Fol* at the world level [1,2]. From studies on gene knockout of Six gene, researchers have discovered the effector gene responsible for pathogenicity in tomato as *Six1* (*Avr3*), *Six3* (*Avr2*), *Six5* and *Six6* are required for full pathogenicity [3]. Many researchers have reported that *Six* was uncovered from xylem sap, it has been said to be secreted in xylem, with *Six* proteins. *Avr2* (*Six3*) localized in internal plant cells as it suppresses PAMP-triggered immunity (PTI) [4]. In addition, each of the four effector knockout strains altered the abundance of a subset of differentially accumulated proteins (DAPs). *Fol* effectors affect the composition of the tomato xylem sap proteome in both unique and common ways [3]. The *Six1* gene is the first a virulence gene encoding a 30 kDa single peptide protein [5]. In matching the tomato plant resistance gene (I, I-1, I-2 and I-3) to the specific pathogen a virulence gene (*Six*), allowing gene by gene for the disease resistance model [6, 7, 5]. The *Fol Six1* gene encodes cysteine-rich protein that localizes in tomato leaves to evolved fungal-plant gene-by-gene interactions [8, 9, 10, 5]. As well as

the *Fol Six* gene acting as effectors as protein is secreted in the xylem sap responsible for wilting of tomatoes worldwide.

Soil-borne filamentous fungi of the genus *Trichoderma* live a saprophytic way of life. *Trichoderma* spp. is the best inhibitor for *Fusarium oxysporum* f.sp. *lycopersici* [11]. When stressed, *Trichoderma* spp. Launched singaling pathway, G protein captures this signal and converts to MAPK and cAMP pathway as revealed by chitinase and secondary metabolites for plant pathogens [12]. Protease, chitinase and -1-3-glucanase secreted by *Trichoderma harzianum* MTCC 3928 has been shown to inhibit *Fol* [13]. *T. virens*, *T. atroviride, T. asperellum and T. harzianum* and *T. reesei* are also implicated as biocontrol agents due to their mycoparasitic behavior [14,15]. Plant growth and defenses can be compromised by *Trichoderma* spp. be strengthened as far as the antagonistic nature for fungal phytopathogens is involved through mycoparasitic interactions, nevertheless it is the best bioagent for plants. The protein domain is a basic protein unit that evolves independently in structure, folding, and function. It is common for small proteins to consist of only a single domain, while large proteins usually contain multiple domains to perform various cellular functions [16].

Here we would separate the domain of the pathogenic protein from *Fol* and the antagonistic protein from *Trichoderma* spp. for *Fol*, together with the analysis of their relationship to each other in the protein domain of *Fol* and *Trichoderma* spp. In addition, we would isolate the protein domain of *Fol* and *Trichoderma* spp. through the use of bioinformatic tools including such as NCBI, pfam, SMART. This is the first work to provide information on the *in silico* characterization domain of the pathogenic protein from *Fusarium oxysporum* f. sp. *Lycopersici* and antagonistic protein from *Trichoderma* spp.

MATERIAL AND METHODS

Here we have collected two types of proteins from NCBI, one is the pathogenic protein from *Fol* and the antagonistic protein from *Trichoderma* spp., named QHQ73564.1 (Secreted in the xylem 5) , QHQ73563.1 (Secreted in the xylem 5) , QHQ73562.1(secreted in the xylem 3) , QHQ73561.1 (secreted in the xylem 3) , QHQ73560.1 (secreted in the xylem 2), QHQ73559.1 (secreted in the xylem 2), QHQ73558.1 (secreted in the xylem 1), QHQ73557.1 (secreted in the xylem 1), KNB02067.1 (peroxiredoxin Q/BCP) , XP_018240112.1 (peroxiredoxin Q/BCP) , EWZ90377.1 (peroxiredoxin Q/BCP) from *Fusarium oxysporum* f. sp. *Lycopersici*(*Fol*) and QBP34034.1 [*T. asperellum*],QBP34035.1[*T. asperellum*],QBZ39488.1[*T. asperelloides*],QBZ39612.1[*T. atroviride*],QRI61462.1[*T. rugulosum*],QRI61463.1[*T. asperelloides*], KAH0491796.1 [*T. gracile*], KAH0527052.1 [*T. semiorbis*], KAH0524343.1[*T. semiorbis*], KAH0493337.1 [*T. gracile*], QTO31282.1[*T. asperelloides*], QRI61468.1 [*T. harzianum*], QRI61467.1 [*T asperelloides*], QRI61466.1[*T. asperelloides*], QRI61465.1[*T. harzianum*], QRI61464.1[*T asperellum*], QBP34032.1[*T. asperellum*], QBP34031.1[*T. asperellum*], QBP34030.1 [*T. asperellum*], QBP34029.1 [*T. asperellum*], CAJ87103.2[*Trichoderma* sp. RV-2006] from *Trichoderma* spp.

Here, domains were analyzed using NCBI CDD (National Center for Biotechnology Information Conserved Domains Database), pfam database (protein family database) and SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de). In addition, the graphing was done using the method of Chen et al., 2020[17]. In addition, we used MEGA11 (The Molecular Evolutionary Genetics Analysis) [16].

NCBI CDD: In this domain analysis, a protein fasta file from both species (*Fol* and *Trichoderma* spp.) with an e-value of 0.01 was forwarded to CD search. During domain analysis, the job ID was QM3-qcdsearch-2DE8F226823F9AC7-FB9B6FC38479E65 for *Fol* and QM3-qcdsearch-32251BE40514FB91-F58A24F56EB5932 for *Trichoderma* spp. available. The CD search tool serves as a web application to search for conserved domains on multiple protein sequences in a single job as a graphical display of the result for protein [17].

pfam: Worked on the basis of several sequence alignments generated by using hidden Markov models. Here we were presented with a protein sequence generated in the domain at an e value of 0.01. The job ID was shown as QM3-qcdsearch-288BF1997264F177 with data source: CDSEARCH/oasis_pfam for Trichoderma spp. and the job id was QM3-qcdsearch-3DF4F511312A55AC-A03FF17DB2C9B72 for *Fol* [48].

SMART: This is a freely available domain prediction website. In its alignment based on multiple sequence alignment as well as identified homologues based on PSI-BLAST (E<0.001) analysis. All results were constructed according to the hidden Markov model (HMM, E < 0.01), including site as http://smart.embl-heidelberg.de/smart/[19].

Gene ontology was done via blast2go.Domain structures were predicted by using https://sable.cchmc.org/[20,21],this tools have been used for structure characterization of domain. All domain protein interaction was done by using https://string-db.org/, version 11.5 and job id is

https://string-db.org/cgi/network?pollingId= bCtGLbQdEk2A& session Id=bZAIRlgD7rbs& url disam=b2 MNCCw23QUE.

RESULTS AND DISCUSSIONS

In case of pathogenic protein of *Fol*

Here we performed various bioinformatics tools for domain analysis. All bioinformatics results were different. When we performed the NCBI-CDD tool for domain analysis for pathogenic proteins of *Fol*, we analyzed 20 type domains named PRX_BCP, Thioredoxin_like-superfamily, Bcp, Bcp-superfamily, PRX_family, AhpC-TSA, PRX_AhpE_like, bcp, AhpC, AhpC Superfamily, Redoxin, PRX_1cys, PRX_Typ2cys, PRX_like2, PRK13190, PRK13191, PRK13189, PRK10382, PRK13599, PTZ00253. It was generated from the sequence of KNB02067.1, XP_018240112.1, EWZ90377.1 and the remaining sequence was not given a domain as shown in Fig.1. Whenever we analyzed the domain of the pathogenic protein of *Fol* via the pfam database, the domain was only referred to as AhpC-TSA (alkylhydroperoxide reductase thiol-specific antioxidant) and as a redoxin domain. Showing the AhpC/TSA family domains as KNB02067.1, XP_018240112.1, EWZ90377.1 in order. Here in, the redoxin family includes peroxiredoxin, thioredoxin, and glutaredoxin proteins. The Redoxin family shows a defense against pathogenic microorganisms (bacteria, fungi and protozoa). It was shown that the AhpC/TSA family domains in the sequence Prx (peroxiredoxins) are ubiquitous enzymes that use a highly reactive cysteine residue to decompose hydroperoxides and can also perform other functions such as molecular chaperone and phaspholipase activities, which contributes to microbial protection against host defenses, demonstrated an oxidative stress response in fungi during stress[21][22]. As it is, *Fol* survives in the plant.

like KNB02067.1, XP_018240112.1, EWZ90377.1. In addition, the redoxin family includes peroxiredoxin, thioredoxin, and glutaredoxin proteins. The Redoxin family shows a defense against pathogenic microorganisms (bacteria, fungi and protozoa).

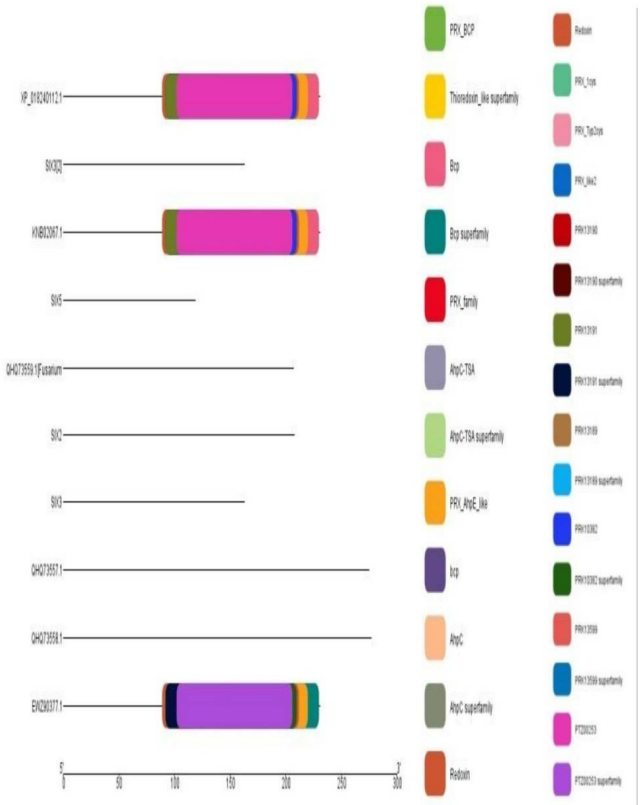


Fig. 1 Pathogenic protein of *Fol* domain protein in visualization analyzed by NCBI, CD search.

SMART tools: This is included two modes normal or genomic. Here we performed a domain analysis in normal mode. Herein, a total of 11 pathogenic Fol proteins used in this tool were analyzed in different low-confidence domains such as **ZnF_CHCC** domain, **Prim_Zn_Ribbon** domain, **PSA** domain, **Knot1** domain , **WR1** domain, **LRRCT** domain, **Agouti** domain , CXC domain, **TRASH** domain, **ACR** domain , **DM6** domain, **RPOLCX** domain, **ZnF_Rad18** domain , **Ephrin_rec_like** domain, **Spc7_N** domain , **Rb_C** domain, **Tet_JBP** domain , **CorC_HlyC** domain, all domains are in Fig. 3 available, it is shown as a phylogenetic tree, the grouping of the domain and the orientation together with the conserved amino acid are presented.

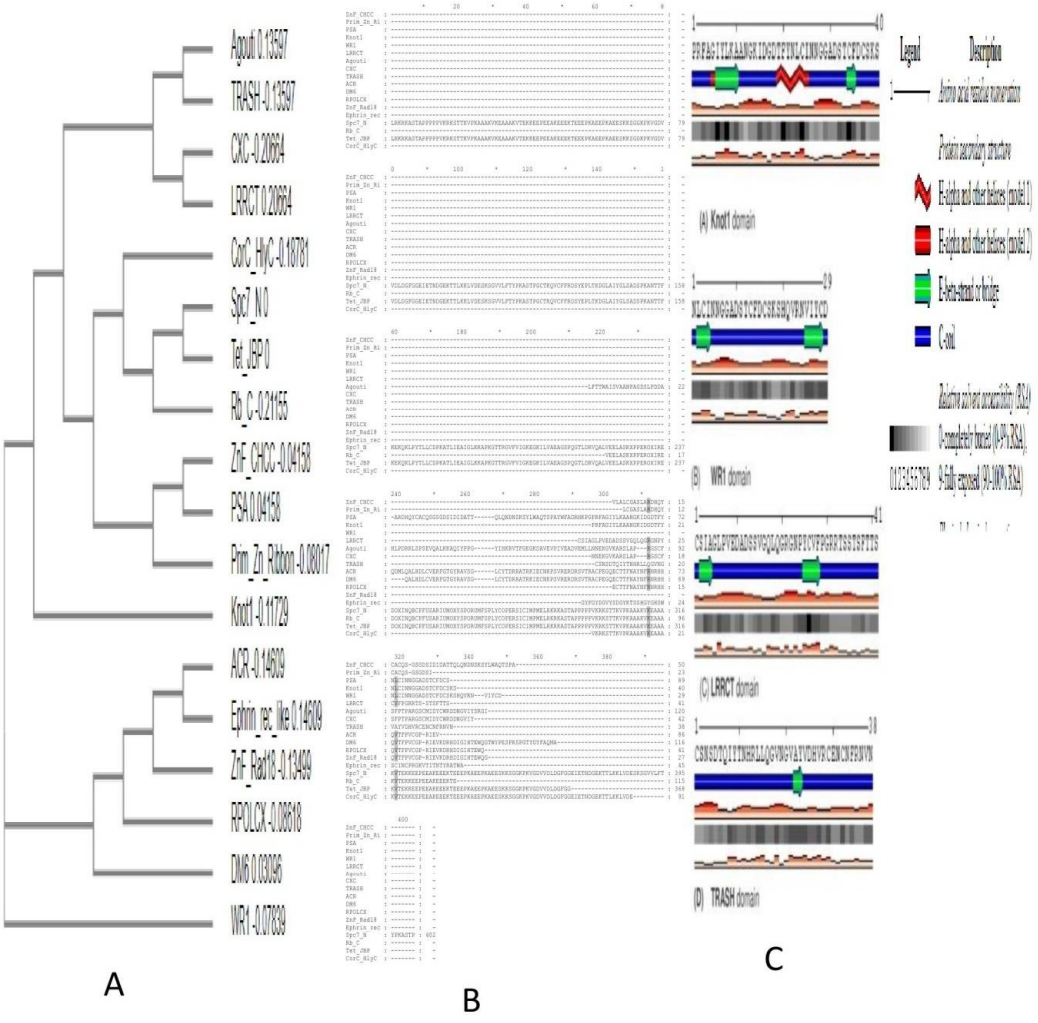


Fig. 3 shows the phylogenetic domain tree in (A). This is a neighbor-joining tree without distance corrections, and the entire *Fol* domain has been shown in (B) with conserved amino acid and orientation,(C) is showing secondary structure of protein domain from *Fol*, this domain direct involved in defenses and growth of *Fol*.

The ZnF_CHCC domain is zinc fingers (Znf), relatively small protein motifs containing multiple finger-like projections that make tandem contacts with their target molecule [24]. They were first identified as a DNA-binding motif in the *Xenopus laevis* transcription factor TFIIIA. The Knot1 domain, known as knottins, is a small protein involved in biological functions such as inhibitors, antimicrobial peptides, and

toxins. Defense reactions (GO: 0006952) and pathogenicity-associated protein domains are shown (Patel et al., 2017).

The WR1 domain is a worm-specific repeat type1, belongs to the MEROPS peptidase inhibitor family 12 and appeared as a cysteine-rich domain, it has been described as a pathogenicity-associated protein domain (Pater, 2017). The LRRCT domain is a leucine-rich repeat C-terminal domain found in viruses to eukaryotes that is significant in the structural framework for the formation of protein-protein interactions[24]. It is represented as LRRs, contained tyrosine kinase receptors, extracellular matrix-binding glycoproteins, receptors, cell adhesion molecules, and virulence factors, all of which are involved in various biological processes, signaling, disease resistance, apoptosis, RNA processing, recombination, transcription, and immune response. Pathogens are not directly inhibited by LRRs. Resistance genes can be identified from their products[25]. The agouti domain released the agouti protein. The CXC domain is a Tesmin/TSO1-like CXC domain. The TRASH domain is a metallochaperone-like domain. As Knot1,WR1, LRRCT, TRASH recognized more significant in pathogenic protein of *FOL*, their secondary structutre has been shown as C-coil and E-beta-strand except knot1,it contained H-alpha (Model1), H-alpha(Model2), two : E-beta-strand,C-coil[26]in fig.3(C). As a result of this analysis, we also identified co-regulated effectors that have a structural relationship[27]. The ACR domain is a cysteine-rich ADAM domain, we can say a cysteine-rich family of proteins. All domains contain a common domain such as a prodomain, the metalloprotease, disintigrin, epidermal growth factor, transmembrane domain. The DM6 domain is a cysteine-rich domain currently specific to Drosophila. The RPOLX domain is the RNA polymerase subunit (X) presented in RNA polymerase I, II and III. The ZnF_Rad18 domain is a Rad18-like CCHC zinc finger. Protein phosphorylation plays a role in most cellular activities mediated by protein kinase and phosphoprotein phosphatases. Protein kinase is a catalytic subunit that results in kinase-specific inhibitors for the treatment of a variety of diseases. The ephrin_rec_like domain is a putative ephrin receptor-like domain with a cysteine-rich region. Spc7_Ndomain is the N-terminus of the Spc7 subunit of the kinetochore-NMS complex. The Rb_c domain is the C-terminal Rb domain. The Tet_JBP domain is an oxygenase domain of the 2OGF-eDO superfamily found in various eukaryotes, bacteria and bacteriophage. The CorC_HlyC domain is a transporter-associated domain involved in magnesium and cobalt efflux. All domains consisted of L, V, R, K type common amino acids and the best alignment was shown from 319 to 328 upto in Fig.3. As we analysed conserve amino acid and domain which support in growth and survive in tomato plants. In addition to the genes for potential pathogenicity, functional categorization suggests that certain cellular processes, such as amino acid and lipid metabolism, cell wall remodeling, protein translocation, and protein degradation seem to be necessary for full pathogenicity to take place in *Fol,* according to Michielse (2009)[28].

In case of antagonistic protein of *Trichoderma* spp.

From NCBI: *Trichoderma* spp. contained many domains in comparisons *Fol*, naming the domain as PksD, PKS_AT, PRK12316, FUM14_C_NRPS-like, A_NRPS_SidN3_like, alpha_am_amid, EntF, PRK12467, AFD_class_I, PRK07768, CT_NRPS-like, PRK12316, PRK08565, Endochitinase, P-loop_NTPNase, translaton. NTPNase Elongation factor 1-alpha, RPB2 (Supplymentary file1). Herein, antagonistic protein from *Trichoderma* spp. for *Fol* retained specific binding sites called PP binding [phosphopantetheine binding site], AMP binding site [chemical binding], pPant-Arm binding site [chemical binding]. The EntF and PksD domain are responsible for the production of secondary metabolites; A_NRPS_SidN3_like is responsible for the adenylation domain (A) of siderophore-synthesizing nonribosomal peptide synthetases (NRPS) that produce siderophore. Thus available domains such as endochitinase, A_NRPS_SidN3_like, EntF and PksD domain in *Trichoderma* spp. may be significant in the prevention of fol, its domain was shown in Fig. 2 [29]. From pfam, CD search (batch) revealed the domain as AMP-binding, AFD_class_I superfamily, acyl_transf_1, condensation, ketoacyl-synt, cond_enzymes superfamily, PP-binding, epimerase, condensation superfamily in two species, named KAH0491796. 1 [*T. graceful*] and KAH0527052.1 [*T. semiorbis*], its domain is shown in Fig. 2 (Pfam domain list in Supplementary file4).

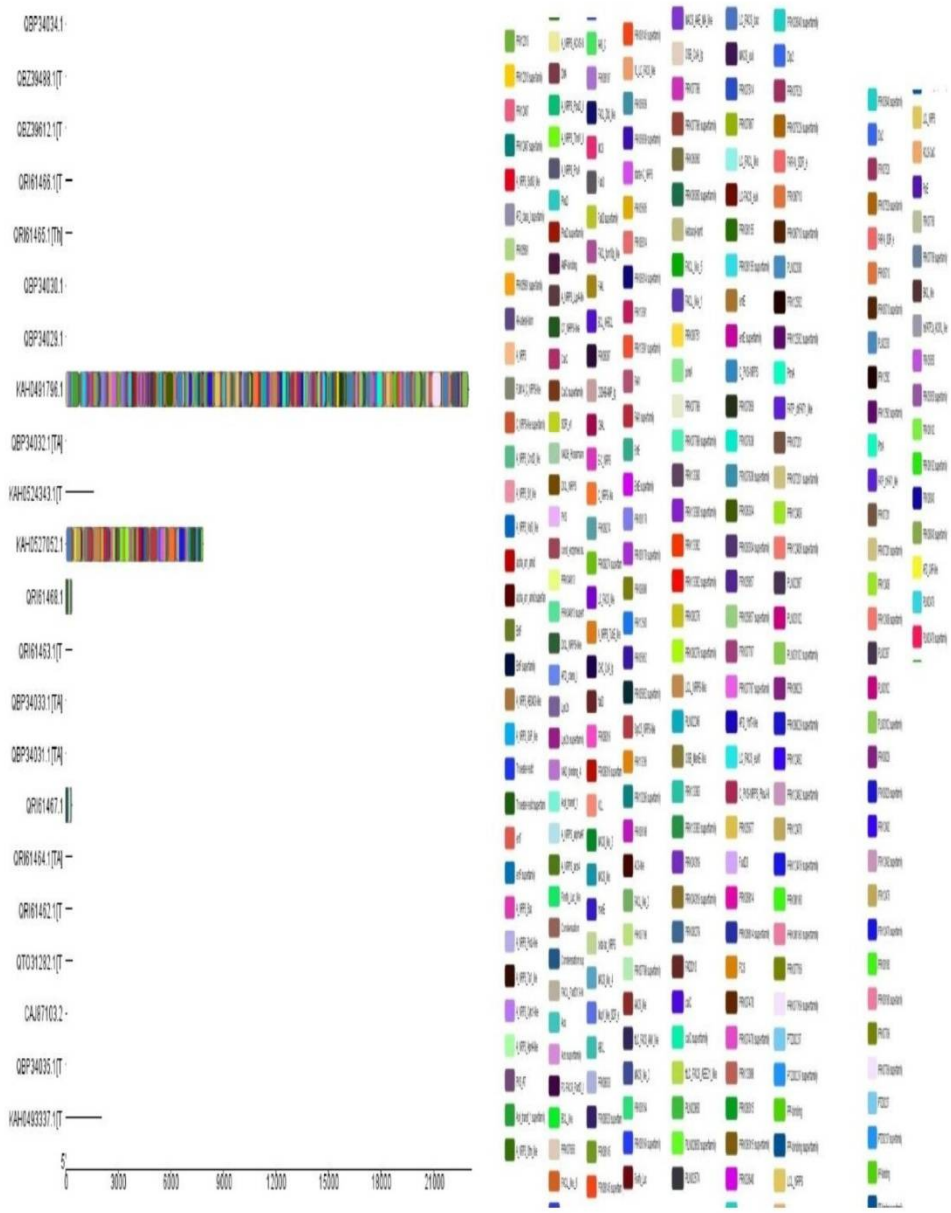


Fig.2 Representation of the Conserve domain of *Trichodermas* spp. is visualized along with its ligand, which has been shown in different colors.

But in the case of *Trichoderma* spp. we analyzed the domain result at two levels, one with high confidence level and the other with low confidence level from SMART (Domain list from SMART (*Trichoderma* spp.) 2 and 4). According to the result, high-level PKS_PP and PKS_AT were available, on the other hand, we analyzed different types of low-level domains (Domain list from SMART (*Trichoderma* spp.) in supplementary file2 and 4). Important domain, UDPG_MGDP_dh_Cdomain, is a UDP binding domain and has activity as NAD binding (GO:0051287), oxidoreductase activity, acts on CH-OH group of donors, NAD or NADP as acceptor (GO:0016616). The CPDc domain is a catalytic domain of ctd-like phosphatases, the essential protein serine phosphatase (EC 3.1.3.16) in yeast. This protein contains a DxDx(T/V) motif followed by four hydrophobic residues typical of metal-dependent phosphohydrolases and

phosphotransferases [30][31]. The PAC domain is the motif C-terminal to PAS motifs (likely contributing to the PAS structural domain), responsible for bacteria and sensors for oxygen and redox [32]. Figure 4 shows the phylogenetic domain tree and its conserved amino acids such as E,L,Q,I,W,A,F,M,G,T,V,D,F,P,R,S are shown in light black color. Protein domain secondary structure has been shown in fig. 4, the diagram shows the letter of the amino acid. Secondary structure mostly contained helix and C-coil structure compare to E-beta sheet. If results from *Trichoderma* spp. Domain was compared where PKSs type domain was mostly available high or large level and other domain was low and small. Furthermore, we analyzed the same result across NCBI, SMART and pfam database. Three types are classified as Type I PKS, Type II, Type III (Sabatini et al., 2018). In general, Type I PKSs use all of the enzymes needed for a cycle of bento chain elongation and processing, and can be modular (most common in bacteria) or iterative (most common in fungi). Yao et al. (2016) reported two polyketide synthase genes from *Trichoderma harzianum* 88 during mycoparasitism, one is pksT-1 (5669 bp) and the other is pksT-2 (7901 bp), which showed characteristics of type I fungal PKS [16]. PKS and NRPS is a multi-enzyme domain that produces numerous metabolites. Subunits undergo self-assembly to ensure correct domain organization for product biosynthesis, with the N- and C-termini of each subunit, known as docking domains (DDs), according to Smith et al. (2021). Carrier protein domains used from PKS (acyl carrier protein (ACP)) and NRPS (peptidyl carrier protein (PCP)), post-translationally modified by attachment of the Ppant(4-phosphopantetheine) group [35]. Polypeptide biosynthesis is possible from the head and tail condensation of acyl and malonyl derived thioester moieties. Modular PKSs have been divided into two classes of cis-AT and trans-AT PKSs, cis-AT bearing an acyltransferase (AT) domain and trans-AT PKSs bearing a domain lacking acyltransferase (AT). PKS and NRPSs responsible for the biosynthesis of polyketide and non-ribosomal peptide natural products, respectively. It is significant in inhibiting *Fol* and plant pathogens. Type I PKS is important for the prevention of *Fol*. The PKS family is a class of megasynthases that catalyze the Claisen decarboxylation of several short carboxylic acid precursors, where the starter unit is the first unit and the extender units are the following [36][37][38]. PKS_AT domain: enzyme such as CoA acyl-carrier protein transacylase (EC2.3.1.39), eukaryotic fatty acid synthase (EC2.3.1.85), polyketide synthase 6-methylsalicylic acid synthase (EC 2.3.1), biosynthesis of patulin and conidia green pigment synthase (EC2.3.1) are functional in their PKS_PP (phosphopantetheine) domain: Pantethein-4-phosphate is the prosthetic group of the acyl carrier protein (ACP) in the multicomplex enzyme, where it serves as a swinging arm for the attachment of activated fatty acids [37,39,40]. HWK_HK domain: It retained the HWE histidine kinase involved in the two-component system. This family conserved H residues. The two-component signaling system enables bacteria to recognize and respond to environments, stressors and growth conditions. Its function has been conferred as protein histidine kinase activity (GO:0004673). Endochitinase 42 released from *T. harzianum*, which also has another name as chitinase (EC 3.2.1.14). Endochitinase42 belongs to the family of glycosyl hydrolases18 and the pfam ID is PF00704. The enzyme acts as an antifungal and shows antagonistic activity as well as mycoparasitism for plant pathogens [41,42,43]. Proteasome_A_N domain: It is conserved in the A subunit of the proteasome complex proteins. In its involved function as ubiquitin-dependent protein degradation process (GO:0006511) and proteasome core complex, alpha subunit complex (GO:0019773). Antimicrobial21 Domain: It is known as a plant antimicrobial peptide that contains an alpha-helical hairpin fold stabilized by two disulfide bonds. It is important in the defense reaction to fungi (GO: 0050832), it has been described as a pathogenicity-associated protein domain [23]. PKS_ER domain (Enoylreductase): It is a type of polyketide synthases that releases many secondary metabolites with multifunctional enzymes. Its significant oxidoreductase activity (GO: 0016491).

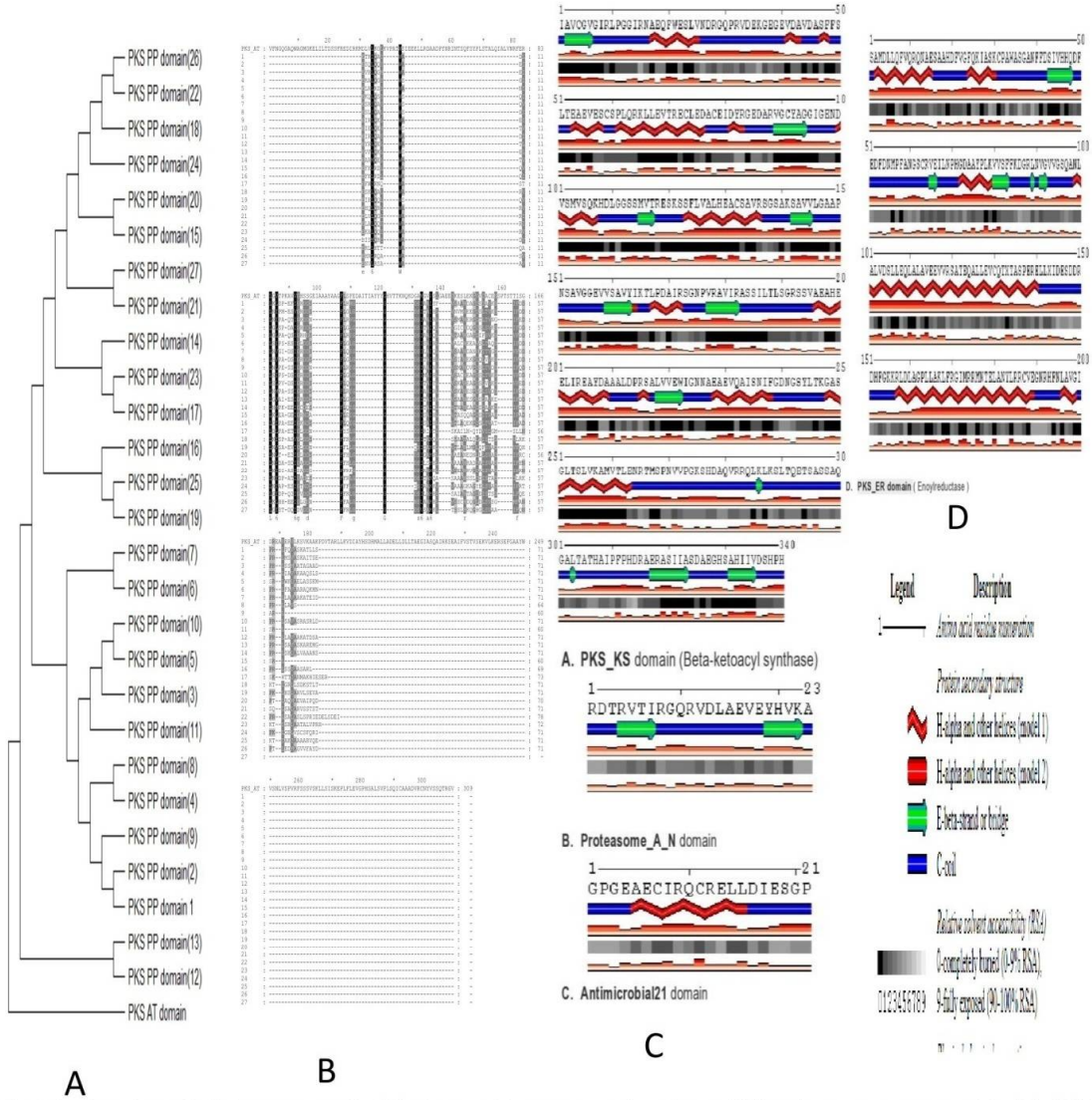


Fig.4 is Showing of phylogenytree in (A) along with conserved amino acid has been represented in (B). (C) is showing secondary structure of protein domain from *Trichoderma* spp.(A) is showing **Evolutionary relationships of taxa :**The evolutionary history was inferred using the Neighbor-Joining method (Saiton and Nei (1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein,1985) (next to the branches). The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling(1965)) and are in the units of the number of amino acid substitutions per site. This analysis involved 28 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 309 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

Gene ontology of antagonistic protein of *Trichoderma* spp.

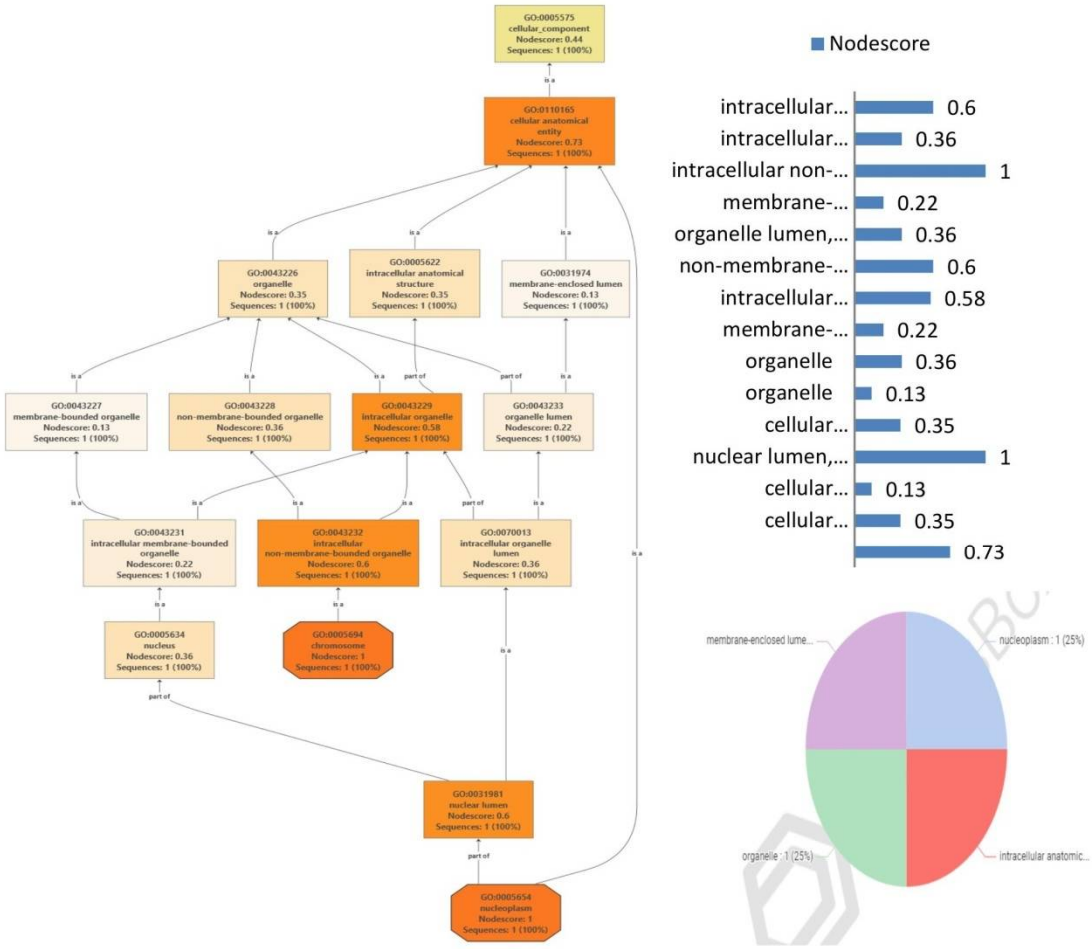


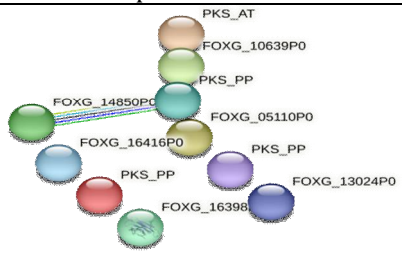
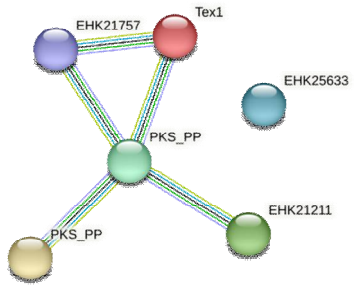
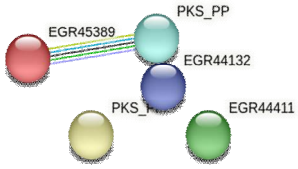
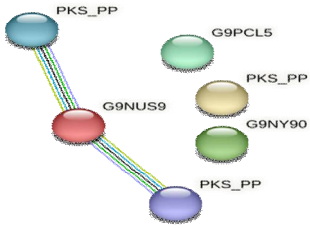
Fig.5 (A) is showing relationship of gene ontology (GO) via node,(B) and (C) is showing cellular component (CC)by using bar and pie graph.

Here in, we were analyzed Molecular function (MF), Biological function (BF), Cellular function (CF). Fig. 5(A) and (B) is showing relation between GO and cellular component of percentile graph are showing in fig.5(C) which is showing equal distribution of antagonistic protein of *Trichoderma* spp.. Ligase, RNA-directed RNA polymerase; DNA-directed RNA polymerase and DNA-directed RNA polymerase are included as enzyme. In this enzyme code is represented as EC:6 ;EC:2.1.1, EC:2.7.7.6, EC:2.7.7.48;EC:2.7.7.6. However, we were also analysed different function as non-ribosomal peptide synthetase [*Trichoderma arundinaceum*], NRPS protein [*Trichoderma parareesei*], non-ribosomal peptide synthetase [*Trichoderma citrinoviride*], non-ribosomal peptide synthetase [*Trichoderma virens* Gv29-8], NRPS protein [*Trichoderma simmonsii*], NRPS protein [*Trichoderma guizhouense*], non-ribosomal peptide synthetase [*Trichoderma arundinaceum*], non-ribosomal peptide synthetase [*Trichoderma longibrachiatum* ATCC 18648], nonribosomal peptide synthase SidD [*Trichoderma reesei* RUT C-30], nonribosomal siderophore peptide synthase Sid2 [*Aspergillus nomiae* NRRL 13137] with GO:0003824, GO:0031177, GO:0044249, GO:0016874, and class V chitinase [*Trichoderma virens*], glycoside hydrolase family 18 protein [*Trichoderma virens* Gv29-8], endochitinase, partial [*Trichoderma virens*], glycoside hydrolase family 18 protein [*Trichoderma asperellum* CBS 433.97], endochitinase ech2 [*Trichoderma*

koningiopsis], glycoside hydrolase family 18 protein [*Trichoderma atroviride* IMI 206040], endochitinase 1 precursor [*Trichoderma harzianum*] with GO:0008152, GO:0004568, GO:0008061, GO:0006032, GO:0000272, GO:0004553, GO:0005576, GO:0005975, GO:0016787, GO:0016798. All its domain has been shown antagonistic properties against *FOL,* as well as we can say that *Trichoderma* spp. shows mycoparasitic behavior for *FOL*.

Protein-protein interaction (PPI)

Here, we examined the interactions among antagonistic protein of Trichoderma spp. and pathogenic protein of Fol domain. According to the findings, T. virens' protein domain shown the best interaction, while T. reesi and T. atroviride both demonstrated PPI but no interaction with other Trichoderma spp. PKS PP and FOXG 14850PO which is the pathogenic gene of Fusarium oxysporum, interacted in Fusarium oxysporum [48]. Table.1 displays the PPI for each domain.Table.1 List of domain interaction.

Fungus name	Job id	Interaction pic.
Fusarium oxysporum (PPI enrichment p-value: 0.13) *your network does **not** have significantly more interactions than expected*	https://string-db.org/cgi/network?taskId=bidg7zU1gioO&sessionId=bwUnY37FShAn	
Trichoderma virens (PPI enrichment p-value: 0.00331) *your network has significantly more interactions than expected*	https://string-db.org/cgi/network?taskId=bW3YnFcsowD7&sessionId=byuThphGfbmd	
Trichoderma reesei (PPI enrichment p-value: 0.337) *your network does **not** have significantly more interactions than expected*	https://string-db.org/cgi/network?taskId=bKRfjUQOrEat&sessionId=bRcdlbsLKLAB	
Trichoderma atroviride (PPI enrichment p-value: 0.225) *your network does **not** have significantly more interactions than expected*	https://string-db.org/cgi/network?taskId=bMr2AQK4YO2b&sessionId=ba2LtoadUqtM	

Here, *Trichoderma atroviride* and *Trichoderma virenus* demonstrated two functions: phosphopantetheine binding (GO: 0031177), ligase activity, and a biological process (pathogenesis, GO: 0009405). (GO: 0016784). The biosynthesis of secondary metabolites is shown by KEGG pathway, map01110. Polyketide synthase complex localisation was within the cell. But in the case of *T. ressi*, it only displayed ligase activity and molecular function phosphopantetheine binding (GO: 0031177). (GO: 0016784). Numerous investigations have documented various *Trichoderma* spp. domains that are important in the suppression of *FOL*. According to Poveda et al. (2019), Kelch domain protein was discovered in *Trichoderma harzianum* T34 and plays a crucial role in *Trichoderma*-plant interactions as a result of root colonisation and systemic defence in Brassicaceae plants. Under abiotic stress, *Trichoderma harzianum* T34 exhibits overexpression of the Thkel1 gene. Under stressful circumstances, *Saccharomyces cerevisiae* zinc-binding proteins improve zinc absorption efficiency [50][51]. In bean plants, the protein Epl-1 causes the production of defence genes [52]. After thorough analysis, it has been determined that the domains of *Fol*

and *Trichoderma* spp. are important in the development and progression of disease. The Sm1 domain from *T. virens*, which offers protection against the leaf pathogen *Colletotrichum* sp., was reported by Djonovi et al. in 2006[53]. According to Kumar et al. (2012)[30], *Trichoderma* spp. are *FOL* inhibitors. Due to their properties, *Trichoderma* spp. are utilised in industry and as a biocontrol agent in agriculture. *Fusarium oxysporum* f. sp. *lycopersici* FOXG gene will be inhibited by *Trichoderma* spp. PKS domain.

CONCLUSION

According to the result, the SMART tool returned the best protein domain as one outcome has a high confidence level and another a low confidence level, compared to NCBI, pfam. Since we concluded that *Fol* contains some specific protein domains, ZnF_CHCCdomain, Prim_Zn_Ribbondomain, PSAdomain, Knot1domain, WR1domain, LRRCTdomain, Agoutidomain, CXC domain, TRASHdomain, ACRdomain, DM6domain, RPOLCXdomain, ZnF_Rad18domain, Ephrin_rec_likedomain, Spc7_Ndomain, Rb_Cdomain, Tet_JBPdomainat, CorC_HlyCdomainat low level so that it could suppress the plant's innate immunity because plants such as plant pathogens grow easily. On the other hand, *Trichoderma* spp.comprised special domain including PKS_AT domain, PKS_PP domain, HWK_HK domain, endochitinase, proteasome_A_N domain, antimicrobial21 domain, PKS_ER domain, all metabolites released from the domain and enzymes involved in *Fol* killing. Secondary structure of FOL retained maximum C-coil and E-beta sheet, and other hand *Trichoderma* contained mostly H-helix and C-coil in comparision E-beta sheet. After, GO analysis and PPI interaction suggested to antagonistic property of *Trichoderma* spp against plant pathogens. As we can understand, all characteristics of this domain of *Trichoderma* spp., occurring in antagonistic nature owing to released metabolites from the accessible domain, can be significant in the inhibition of *Fol*.

Abbreviations

Six: Secreted in the xylem
Fol: Fusarium oxysporum f.sp. *lycopersici*
T: Trichoderma
NCBI-CDD: National Center for Biotechnology Information Conserved Domains Database
pfam : Protein families database
SMART: Simple Modular Architecture Research tool
MEGA : The Molecular Evolutionary Genetics Analysis
PPI: Protein-protein Interaction

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