

Trichoderma Genome to Genomics: A Review

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Abstract

Trichoderma species are widely used in agriculture as biopesticides. These fungi reproduce asexually by production of conidia and chlamydoconidia and in wild habitats by ascospores. *Trichoderma* species are well known for their production of enzymes called Cell Wall Degrading Enzymes (CWDEs). 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 pathogens. These biocontrol genes can be cloned in huge amounts and can be used on large scale for commercial production. Some *Trichoderma* genes are also helpful in providing resistance to the biotic and abiotic stresses such as heat, drought and salt. The major biocontrol processes include antibiosis, mycoparasitism and providing plant nutrition.

Keywords: *Trichoderma*; Biocontrol genes; Cell wall degrading enzymes

Abbreviations: ITS: Internal Transcribed Spacer; GOLD: Genome Online Database; ISTH: International Sub-commission on *Trichoderma* and *Hypocrea* Taxonomy; ICTF: International Commission on the Taxonomy of Fungi; CWDEs: Cell Wall Degrading Enzymes

Introduction

The genus *Trichoderma* is commonly found in soils and on decaying wood and vegetable matter. Strains of *Trichoderma* are rarely associated with diseases of living plants, although an aggressive strains of *Trichoderma* causes a significant disease of the commercial mushroom and soil borne pathogens. Samuels [1] provides a comprehensive review of the biology of *Trichoderma*, and of the technological exploitation of *Trichoderma* species for enzyme production and biological control. In *Trichoderma* species sexual reproduction is not present and are believed to be mitotic and clonal. The nomenclature of these fungi is complicated because of their pleiomorphism -that is, some of them can exist in two morphologically and physiologically different stages. The sexual (teleomorphic) stage is known by the generic name *Hypocrea*, whereas the asexual (anamorphic or mitosporic) stage is called *Trichoderma*; here, we refer to the genus collectively as *Hypocrea/Trichoderma*. However, despite these significant advances in our knowledge of the genus, the taxonomy of *Trichoderma* is still incomplete, and the distinction of species in the genus *Trichoderma* remains problematic. A refined classification and identification is necessary for predictive indications about ecology.

Taxonomic history

Although the genus *Trichoderma* has been known since 19th century. Its association with teleomorphs in *Hypocrea* Fr. was recognized by the Tulasne brothers in 1865, its taxonomy has remained obscure until recent decades. Bisby [2] thought that the morphological variation could be ascribed to a single species, *T. viride*. The first serious attempt to morphologically distinguish species, or "species aggregates", was made by Rifai [3], who was aware that the nine taxa he distinguished were not biological entities correlated with single teleomorph species. These and a few additional species described subsequently were keyed out by Domsch et al. [4]. Teleomorph connections were established by means of ascospore isolates by Dingley [5], and by Webster and coworkers (Rifai and Webster, Webster and Rifai). In Japan, a wealth of teleomorphs was studied thoroughly and described with cultural and anamorph characters by Doi [6-7], but unfortunately no cultures

have been preserved from this study. No further morphological differentiation of the anamorphs was attempted by Doi and Doi. The most detailed morphological studies of the anamorphs were carried out by Bissett [8-12], who now distinguishes about 21 taxa in sect. *Pachybasium* and seven in sect. *Longibrachiatum*, while the remaining sections have not yet been treated in a comparable way. Such studies show that the delimitation of biological species is extremely difficult in this genus on morphological grounds alone.

Other taxonomic methods supplementary to morphology include studies of secondary metabolites, which show a great diversity in this genus [13]. Physiological features detectable in microtiter plates may eventually provide a useful system for identification. Isoenzyme profiles have been used as an effective taxonomic tool [14]. Molecular data, particularly sequences of the ITS region of ribosomal DNA and fingerprinting techniques, have in recent years allowed the finest resolution of taxonomic entities [15-17].

Trichoderma genetics

The genome size of filamentous fungi is usually small. They have a haploid nucleus. The estimated genome sizes and chromosome numbers of *Trichoderma* spp. range from 31 to 39 Mb and from 3 to 7, respectively. The size of chromosome differs among the species. International meetings devoted to *Trichoderma* are organising in whole world. In October 2010 a ARD (US-Israel Binational Research and Development Fund) workshop, 'Molecular mechanisms and applications of biocontrol in agriculture', was held at the Technion, Israel Institute of Technology, Haifa, Israel. This workshop was organised after the release of the sequenced genomes of *T. atroviride* and *T. virens*, which, together with *T. reesei*, gave novel insight into the evolution of mycoparasitism [18-26].

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Genomics of *Trichoderma*

Among different species of *Trichoderma* *T.reesei* is the species whose genome is completely sequenced. From industrial point of view this strain is very important, as this strain has a large capacity to produce cellulose and hemicelluloses enzymes. Genome size of *T. reesei* is 33 Mb and it has seven chromosomes. Apart from *T.reesei* there are some other species whose genome is sequenced completely and specific characters of their sequenced genomes are listed below:

Genomic analysis of the important genes/nucleotides involved in biocontrol mechanism in *Trichoderma spp.* by bioinformatics tools

ISTH (International Sub-commission on *Trichoderma* and *Hypocrea* Taxonomy), a Sub-commission of ICTF (International Commission on the Taxonomy of Fungi), hosts an online method for the quick molecular identification of *Hypocrea/Trichoderma* species based on an oligonucleotide barcode: a diagnostic combination of several oligonucleotides (hallmarks) specifically allocated within the Internal Transcribed Spacer 1 and 2 (ITS1 and 2) sequences of rDNA repeat. It helps in identifying specific strains of *Trichoderma* by

comparing the sequence with the database by locating Genus Specific Hallmarks (GSH).

Once the strains are isolated in wet lab and their morphology is studied based on which the strain identification is done, the identification of isolated strains is done and validated at the ISTH website. As ISTH is solely dedicated for the identification of different strains of *Trichoderma* and *Hypocrea* species based on ITS sequences and other taxonomical data, the strains under study in this project are also validated through ISTH database.

The nucleotide sequences (submitted and retrieved from NCBI) of all seven strains of *Trichoderma* species are analyzed through *TrichOKEY* 2 program for their validation post molecular identification. This has confirmed the selected sequences as specific strains of *Trichoderma* species. A set of 5 oligonucleotide sequences which are present in all known *Hypocrea/Trichoderma* ITS1 - 5.8S RNA - ITS2 sequences, is used in combinations to identify the species at generic level.

TrichoMARK v. 1.0 is used for the detection of multiloci phylogenetic markers. It detects the presence of Internal Transcribed Spacer (ITS) regions in the entered sequences.

Features	<i>T. reesei</i>	<i>T. virens</i>	<i>T. atroviride</i>	<i>T. harzianum</i>	<i>T. asperellum</i>	<i>T. longibrachiatum</i>	<i>T. ctrinoviride</i>
Genome size (Mb)	34.1	39	36.1	40.98	37.46	32.24	33.48
No of predicted genes	9129	12427	11863	14095	12566	10792	9397
Glycosyl hydrolases	Chitinases	23	41	34	NA	NA	NA
	Glucanase	15	18	18	NA	NA	NA
Secondary metabolites Biosynthesis, transport and catabolisms (KOG)	262	440	349	438	358	253	285
PKS	11	18	18	NA	NA	NA	NA
NRPS	10	28	16	NA	NA	NA	NA
PKS-NRPS	2	4	1	NA	NA	NA	NA
S SCPs	260	319	301	NA	NA	NA	NA
Xenobiotics bidegradation and metabolisms (KEGG)	327	519	453	610	432	232	359
Mating types	MAT1-2	MAT1-2	MAT1-2	MAT1-2	MAT1	MAT1-1	MAT1-2

Table 1: A summary of some features of the sequenced genomes.

Name of gene	Source Organism	Function
<i>Tvsp1</i>	<i>Trichoderma virens</i>	This gene encodes for serine protease. <i>Rhizoctonia solani</i> which affects the cotton seedlings has been controlled biologically by serine protease.
<i>tri5</i>	<i>Trichoderma harzianum</i>	This 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.
<i>TgaA</i> and <i>TgaB</i>	<i>Trichoderma virens</i> ,	This gene exhibits antagonist activity against <i>R. solani</i> and <i>Sclerotium rolfsii</i>
<i>ThPG1</i>	<i>Trichoderma harzianum</i>	This gene encodes for endopoly- galacturonase. This enzyme involves in the cell wall degradation of the pathogens like <i>R. solani</i> and <i>P. ultimum</i>
<i>Th-Chit</i>	<i>Trichoderma harzianum</i>	This gene is responsible for the antifungal activity in transgenic tobacco plant.
<i>tri5</i>	<i>Trichoderma brevicompactum</i>	This gene helps in the production of Trichodermin which shows antifungal activity against <i>S. cerevisiae</i> , <i>Kluyveromyces marxianus</i> , <i>Candida albicans</i> , <i>C. glabrata</i> , <i>C. tropicalis</i> and <i>Aspergillus fumigates</i> .
<i>erg1</i>	<i>Trichoderma harzianum</i>	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.
<i>TvGST</i>	<i>Trichoderma virens</i>	This gene is responsible for cadmium tolerance
<i>Thkel1</i>	<i>Trichoderma harzianum</i>	This gene codes for putative kelch-repeat protein which helps in regulating the glucosidase activity and enhances tolerance to salt and osmotic stresses in <i>Arabidopsis thaliana</i> plants
<i>egl1</i>	<i>Trichoderma longibrachiatum</i>	This gene showed biocontrol activity against <i>P. ultimum</i> in damping- off of cucumber
<i>qid74</i>	<i>Trichoderma harzianum</i> CECT 2413	This gene plays a significant role in cell protection and provide adherence to hydrophobic surfaces that helps the fungus in mycoparasitic activity against <i>R. solani</i> pathogen
<i>Taabc2</i>	<i>Trichoderma atroviride</i>	This gene has a significant role in ATP Binding Cassette (ABC) transporter in cell membrane pump that helps in the mycoparasitic activity
<i>tac1</i>	<i>Trichoderma virens</i>	This gene has its role in mycoparasitic activity against <i>R. solani</i> and <i>P. ultimum</i>
<i>TrCCD1</i>	<i>Trichoderma reesei</i>	This gene involves in carotenoid metabolism that helps in the development of conidiospores and hyphal growth in <i>T. reesei</i>

Table 2: List of biocontrol genes involved in Biocontrol Mechanisms.

Trichoderma Biocontrol genes and their function

Trichoderma species are used as biocontrol agents in all over the world. This genera have several biocontrol genes that are involved in the biocontrol mechanisms of (Pratibha sharma et al. 2011). Some major kinds of genes that are involved in biocontrol action are protease, chitinase, glucanase, tubulins, cell adhesion proteins and stress tolerant genes. These genes are responsible for cell wall degradation, hyphal growth, stress tolerance and parasitic activity. For example chitinase is responsible for the breakdown of glycosidic bond, xylanase for hemicellulose etc.

Due to their biotechnological and biological importance the genome sequencing of fungal genomes is under process. About near 500 fungal genomes have been sequenced. The information of all the sequenced genomes is available on Genome Online Database GOLD (<https://gold.jgi-psf.org/cgi-bin/GOLD/bin/gold.cgi>). The Fungal Genome Initiative at the Broad Institute currently have around 50 fungal genomes, mainly from the Ascomycota, and includes model organisms such as *Neurospora* and *Aspergillus* species, but also extends to the genomes of several basidiomycetes, chytrids, and at least one mucormycete (<http://www.broadinstitute.org/scientific-community/science/projects/fungal-genome%20initiative/fungal-genome-initiative>). The mycorrhizal genome initiative, designed to sequence and analyze the genomes of ascomycetes and basidiomycetes that function in symbiotic relationship with woody shrubs and trees. After compilation, this data will provide a framework for understanding the biology of these organisms and exploring their applications in reforestation and maintenance of ecological biodiversity. The fungal kingdom contributes for around more than 15% of species richness found in the major group of microorganisms. The genome analysis of the 28S rRNA is the best used techniques for determining the fungal diversity.

Conclusion

Research data accumulated in the last few years have explore many new applications of fungal species into agriculture and biotechnology sector. These fungal species have the ability to change plant metabolism and provide resistance to abiotic and biotic stress. The genes present within the *Trichoderma* species have been found to have many biocontrol activities. This fungus secretes several cell wall degrading enzymes. These enzymes are used by transgenic plant cell to destroy phytopathogens. In an experiment performed by Liu et al. fungal cell wall degrading enzyme genes were isolated from the fungus *Trichoderma atroviride* and inserted into the rice genome via Agrobacterium-mediated transformation endochitinase ech42, exochitinase nag70 and exo-1,3-glucanase gluc78 were excised from *Trichoderma atroviride* and were used to prepared plasmids containing different combinations. Inoculation of the plants with *R. solani* resulted in a high sheath blight resistance rate in plants that received the ech42 gene. *Trichoderma* species are all well known for their biocontrol properties in the whole world. This biocontrol activity is well known by the characterization and isolation of the specific genes which are involved in this mechanism. The phytopathogens cause a major loss in the agriculture sector. So, farmers use hazardous chemicals for the prevention of these phytopathogens. These chemical pesticides are very harmful for the environment as the leave harmful residues in the soil, affecting the soil fertility. By increasing the use of biocontrol agents this problem can be overcome. The genes isolated from these biocontrol agents found to play an important role in the biocontrol activity. With help of genetic engineering still more number of genes should be discovered for the benefit of future generation.

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