Trichoderma Research in the Genome Era

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Abstract

Trichoderma species are widely used in agriculture and industry as biopesticides and sources of enzymes, respectively. These fungi reproduce asexually by production of conidia and chlamydospores and in wild habitats by ascospores. *Trichoderma* species are efficient mycoparasites and prolific producers of secondary metabolites, some of which have clinical importance. However, the ecological or biological significance of this metabolite diversity is sorely lagging behind the chemical significance. Many strains produce elicitors and induce resistance in plants through colonization of roots. Seven species have now been sequenced. Comparison of a primarily saprophytic species with two mycoparasitic species has provided striking contrasts and has established that mycoparasitism is an ancestral trait of this genus. Among the interesting outcomes of genome comparison is the discovery of a vast repertoire of secondary metabolism pathways and of numerous small cysteine-rich secreted proteins. Genomics has also facilitated investigation of sexual crossing in *Trichoderma reesei*, suggesting the possibility of strain improvement through hybridization.

INTRODUCTION

Endophytes: microbes that live within plants without causing apparent harm; the association is often beneficial to plant fitness and survival

Secondary metabolites: metabolites or natural products produced by an organism but not essential for survival that have broad functions, such as signaling or defense

Mycoparasite: a fungus living in association with and deriving nutrients from another fungus

Induced systemic resistance (ISR): priming by infection with a pathogen or beneficial microorganism

Systemic acquired resistance (SAR): response of the whole plant to infection that may be local; analogous to animal innate immunity

Symbiosis: association of two or more organisms that is mutually beneficial

As ubiquitous and often predominant components of the mycoflora in native and agricultural soils throughout all climatic zones, *Trichoderma* species play an important role in ecosystem health (64). Adapted to virtually every ecosystem, these fungi live in marine and terrestrial sites. They colonize aboveground and belowground plant organs and grow between living cells (endophytes), and they appear in plant litter, soil organic matter (saprophytes), and mammalian tissues (human pathogens). However, the ability of these fungi to sense, invade, and destroy other fungi has been the major driving force behind their commercial success as biopesticides (more than 60% of all registered biopesticides are *Trichoderma*-based) (136). These fungi not only protect plants by killing other fungi and certain nematodes but induce resistance against plant pathogens, impart abiotic stress tolerance, improve plant growth and vigor, solubilize plant nutrients, and bioremediate heavy metals and environmental pollutants (53, 78, 81, 126). In addition, this genus comprises fungi that produce secondary metabolites of clinical significance and enzymes with widespread industrial application. As *Trichoderma* has had a major impact on human welfare, recent genome sequencing projects have targeted seven species: *Trichoderma reesei*, *Trichoderma virens*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderma longibrachiatum*, and *Trichoderma citrinoviride* (**http://genome.jgi-psf.org/programs/fungi**) (44). The genome sequencing of *Trichoderma* species has stimulated the development of systems biological approaches, initiated and enhanced whole-genome expression studies, and provided unique data for phylogenetic and bioinformatic analyses toward understanding the roles of these opportunists in ecosystems. Better understanding of how *Trichoderma* evolved to interact with other fungi and with plants will improve and expand their applications. The ability to attack other fungi, most importantly soilborne plant pathogens, dominated the interest in *Trichoderma* for many years (5, 50, 51, 55, 138). Analysis of the genomes of the mycoparasites *T. atroviride* (teleomorph *Hypocrea atroviridis*) and *T. virens* (formerly *Gliocladium virens*, teleomorph *Hypocrea virens*) compared with the cellulose-degrading, primarily saprophytic *T. reesei* (teleomorph *Hypocrea jecorina*) demonstrated that genes specific to the mycoparasites arose in a common ancestor but were lost from the *T. reesei* lineage (67). Although diverse *Trichoderma* spp. are investigated for their mycotrophy, research with *T. reesei* has mainly focused on production of plant cell wall–degrading enzymes (CWDEs) (68, 120), for which this species has become a model system. If mycotrophy was the original lifestyle of *Trichoderma* species, they also coevolved with land plants to form symbioses with roots. Recent years have witnessed a wave of interest in plant disease resistance [induced systemic resistance (ISR); to some extent, systemic acquired resistance (SAR)] induced by the *Trichoderma*-root symbiosis (52, 53). These plant-centered mechanisms have rivaled mycoparasitism as an explanation for how *Trichoderma* controls plant diseases (78, 126). The combined ability to attack soilborne pathogens while priming plant defenses, however, is what promotes *Trichoderma* as such a promising partner for sustainable management of plant diseases (**Figure 1**). This review focuses on the novel contribution of genomics to the study of *Trichoderma* biology and interactions with other organisms.

LANDMARKS IN *TRICHODERMA* RESEARCH: A HISTORICAL PERSPECTIVE

The genus *Trichoderma* was erected by Persoon in 1794 but captured the attention of agriculturists only after Weindling and his associates showed that one species of the genus can kill other fungi and control plant diseases (141, 142). Research into biological control of insect pests and pathogens slowed after

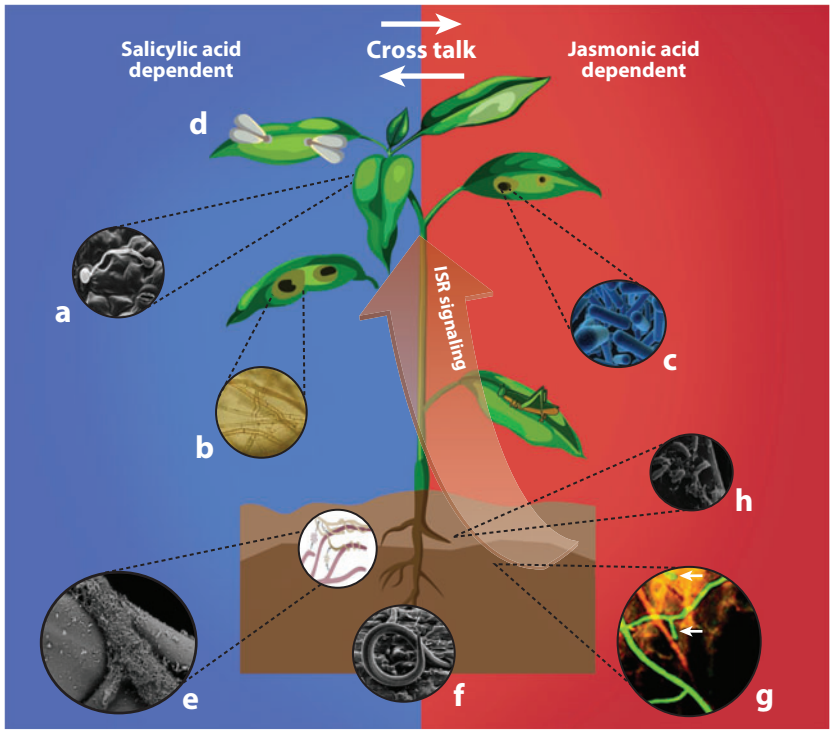


Figure 1

Interactions of *Trichoderma* with plants and plant pathogens. Induction of systemic responses by *Trichoderma* helps protect aerial parts of plants from the attacks of (*a*) biotrophic and (*b*) necrotrophic fungi as well as from (*c*) bacteria and (*d*) insects. Additionally, *Trichoderma* directly attacks (*e*) phytopathogenic fungi and even (*f*) nematodes. Systemic protection of the plant is at least in part due to the colonization of the (*g*) rhizosphere and the (*h*) root system by the beneficial fungus, which triggers induced systemic resistance. Arrows indicate cross communication between elements of the jasmonic and salicylic plant defense response pathways. Design: Mario Ivan Alemán-Duarte, Herrera-Estrella lab. Abbreviation: ISR, induced systemic resistance.

the discovery of synthetic pesticide molecules during/after World War II. Renewed interest in biological pest management was stimulated following the demonstration by environmentalists and ecologists that broad and repeated application of these synthetic molecules could be ecologically harmful (26). However, the first field success of biological control (target *Sclerotium rolfsii*) using *Trichoderma* was not until the 1970s (143). Applied and fundamental research on these fungi has continued unabated since, as evident from a timeline (**Figure 2**), which presents many of the landmark discoveries in the biology and applications of *Trichoderma* in biotechnology and agriculture.

TRICHODERMA GENETICS AND BIOLOGY

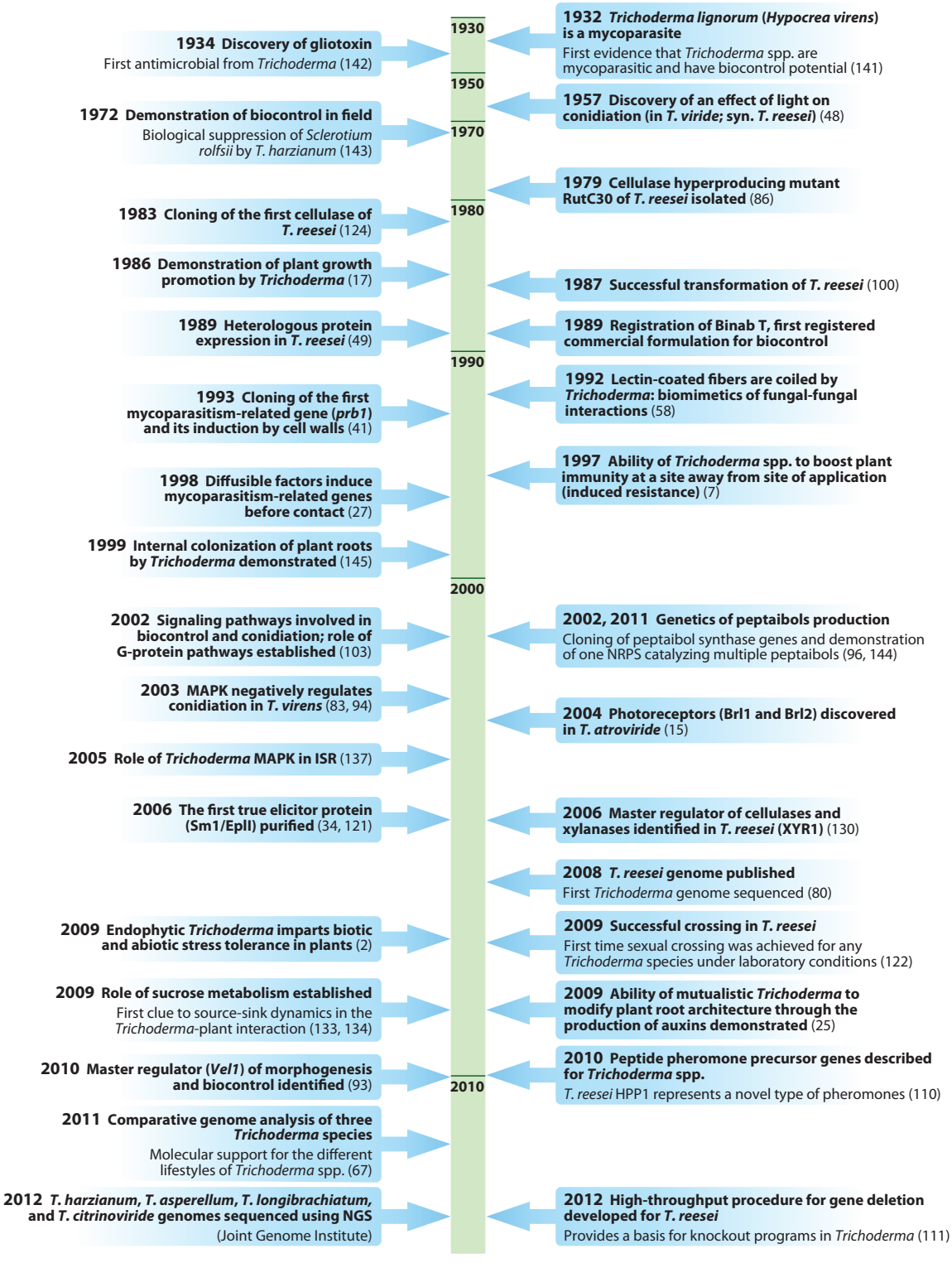
Morphogenesis and Development

The balance between sporulation and hyphal proliferation is critical to the establishment of *Trichoderma* in the soil and rhizosphere (128). Most laboratory strains produce conidia and chlamydospores, and in nature some form ascospores in perithecia. The transition from vegetative growth to conidiation can be triggered in *Trichoderma* by UV-blue light, nutrient deprivation, low pH, or mechanical damage to the mycelium (15, 43, 54, 56, 71). Whether the fungus conidiates or not depends on the cross talk among the pathways involved

Conidia: fungal spores produced asexually, usually differentiated from specialized hyphae (conidiophores)

Chlamydospores: thick-walled hyphae-derived asexual structures important for survival of a fungus

Ascospores: fungal spores produced from the products of meiosis, developing within a sac-like structure (ascus)


1930
1950
1970
1980
1990
2000
2010
1932 Trichoderma lignorum (Hypocrea virens) is a mycoparasite
First evidence that Trichoderma spp. are mycoparasitic and have biocontrol potential (141)
1934 Discovery of gliotoxin
First antimicrobial from Trichoderma (142)
1957 Discovery of an effect of light on conidiation (in T. viride; syn. T. reesei) (48)
1972 Demonstration of biocontrol in field
Biological suppression of Sclerotium rolfsii by T. harzianum (143)
1979 Cellulase hyperproducing mutant RutC30 of T. reesei isolated (86)
1983 Cloning of the first cellulase of T. reesei (124)
1986 Demonstration of plant growth promotion by Trichoderma (17)
1987 Successful transformation of T. reesei (100)
1989 Heterologous protein expression in T. reesei (49)
1989 Registration of Binab T, first registered commercial formulation for biocontrol
1992 Lectin-coated fibers are coiled by Trichoderma: biomimetics of fungal-fungal interactions (58)
1993 Cloning of the first mycoparasitism-related gene (prb1) and its induction by cell walls (41)
1997 Ability of Trichoderma spp. to boost plant immunity at a site away from site of application (induced resistance) (7)
1998 Diffusible factors induce mycoparasitism-related genes before contact (27)
1999 Internal colonization of plant roots by Trichoderma demonstrated (145)
2002, 2011 Genetics of peptaibols production
Cloning of peptaibol synthase genes and demonstration of one NRPS catalyzing multiple peptaibols (96, 144)
2002 Signaling pathways involved in biocontrol and conidiation; role of G-protein pathways established (103)
2003 MAPK negatively regulates conidiation in T. virens (83, 94)
2004 Photoreceptors (Brl1 and Brl2) discovered in T. atroviride (15)
2005 Role of Trichoderma MAPK in ISR (137)
2006 The first true elicitor protein (Sm1/EpII) purified (34, 121)
2006 Master regulator of cellulases and xylanases identified in T. reesei (XYR1) (130)
2008 T. reesei genome published
First Trichoderma genome sequenced (80)
2009 Endophytic Trichoderma imparts biotic and abiotic stress tolerance in plants (2)
2009 Successful crossing in T. reesei
First time sexual crossing was achieved for any Trichoderma species under laboratory conditions (122)
2009 Role of sucrose metabolism established
First clue to source-sink dynamics in the Trichoderma-plant interaction (133, 134)
2009 Ability of mutualistic Trichoderma to modify plant root architecture through the production of auxins demonstrated (25)
2010 Master regulator (Vel1) of morphogenesis and biocontrol identified (93)
2010 Peptide pheromone precursor genes described for Trichoderma spp.
T. reesei HPP1 represents a novel type of pheromones (110)
2011 Comparative genome analysis of three Trichoderma species
Molecular support for the different lifestyles of Trichoderma spp. (67)
2012 T. harzianum, T. asperellum, T. longibrachiatum, and T. citrinoviride genomes sequenced using NGS
(Joint Genome Institute)
2012 High-throughput procedure for gene deletion developed for T. reesei
Provides a basis for knockout programs in Trichoderma (111)

in perception of these environmental cues. Given that many *Trichoderma* species are common soil inhabitants that associate with plant roots, a reflection of the role of light in their life cycle is the induction of conidiation once the fungi reach the soil surface. Under such circumstances, *Trichoderma* must confront the harmful effects of sunlight, yet disperse. Arrested growth, strengthened cell walls and UV-protective pigments make conidia well-suited as dispersal structures. Light also triggers the expression of genes involved in production of compounds that protect *Trichoderma* from photodamage, e.g., photolyases for the repair of DNA damage by UV light, genes for synthesis of pigments that serve as sunscreens, and genes that encode reactive oxygen species (ROS)-scavenging proteins (6) (J. Guzmán-Moreno, A. Flores, L. Brieba, A. Herrera-Estrella; unpublished results; M. García-Esquivel, U. Esquivel-Naranjo, M. Hernandez-Onate, A. Herrera-Estrella, unpublished results). These photoresponses depend on the *blr-1* and *blr-2* genes to encode homologs of the *Neurospora crassa* photoreceptors White Collar 1 and 2 (15, 76). Light-dependent sporulation is less tightly regulated in *T. reesei*, but these photoreceptors and the small PAS-domain photoreceptor ENV1 still have influence (16, 108).

Nutrient deprivation or different nutrient sources are universal signals for conidiation, and light is a determinant for the utilization and/or uptake of specific carbon sources by *Trichoderma* species (132). Several components of the heterotrimeric G-protein and cAMP pathways and two Ras GTPases impact sporulation, often in a light- and nutrient-dependent manner (103, 109, 112, 115, 131, 147). Conidiation by *T. viride* and *T. atroviride* is strongly carbon-source dependent in light and darkness (21, 39). Vegetative growth on different carbon sources is also influenced by light in *T. atroviride*. BLR1 is responsible for carbon source selectivity, but the intensity of the response depends on both BLR proteins (40). These effects of light may also be reflected in the ecological behavior of *Trichoderma* given that carbon sources at the soil surface are different from those associated with roots and buried organic matter. Low pH seems to be a determinant for conidiation, with differences from species to species or even isolate to isolate (14, 129). Choice of biocontrol strains for soils with different pH should take into account, among other factors, how pH modulates growth and conidiation.

Although chlamydospores were discovered in the 1950s, our understanding of their biology and of the genetic basis for formation and proliferation are incomplete. Chlamydospores are produced profusely in liquid submerged culture and are the active propagules in some *Trichoderma*-based commercial formulations (SoilGard™). The advantages of using chlamydospores in formulations are a lower susceptibility to soil fungistasis, a resistance to environmental stress, a greater inoculum potential compared with conidia, and a longer shelf life (98). Mutants with a deletion of the velvet protein Vel1 produce more chlamydospores under nutrient stress compared with the wild type (93). A radiation-induced nonconidiation mutant (M7) also produced more chlamydospores, presumably at the expense of conidiation (89).

Strains of *H. jecorina* were frequently isolated from sexually developing cultures in nature (60), with *T. reesei* considered a clonal derivative (70). The discovery of a peptide pheromone precursor in the genome of *T. reesei* led to the successful crossing of *T. reesei* QM6a with an *H. jecorina* isolate (110, 122). Regulation of the pheromone system of QM6a is altered compared with a sexually competent wild-type strain (117). Investigation of the molecular basis for this defect is in progress and may provide hints as to the possibility of sexual development in other species. The

Pheromone: small peptides acting as messengers for the presence of a potential mating partner; important for sexual development

Figure 2

Timeline of *Trichoderma* research. Abbreviation: ISR, induced system resistance; MAPK, mitogen-activated protein kinase; NGS, next-generation sequencing; NRPS, nonribosomal peptide synthetase.

Mating type: locus in ascomycetes where either of two divergent coding sequences MAT1-1 or MAT1-2 (idiomorphs) are found

Biotrophic: host-parasite interaction where the parasite or pathogen obtains nutrients from living host tissue

discovery of the two mating type loci (MAT1-1 and MAT1-2) enabled induction of sexual development in other *T. reesei* and *H. jecorina* strains from several habitats in the laboratory; however, compatible mating types turned out to not be a guarantee for successful sexual development. Knowledge of the genomic sequences for both mating type loci in *T. reesei* now provides opportunities for rapid screening for compatible partners in other species of the genus. Successful crosses would provide a novel tool for strain improvement, starting with native or previously modified strains.

Initiation of sexual development and ascospore discharge are also regulated by light (122). In darkness, fruiting body formation is delayed, whereas constant light completely inhibits sexual development. Interestingly, the photoreceptors BLR1 and BLR2 influence this process via regulation of fruiting body morphology (18). However, they are not essential for sexual development, whereas the light-regulatory protein ENV1 is needed for female fertility in light. ENV1 regulates, specifically in light, transcription of pheromone receptors and pheromone precursors as well as the mating type transcription factor gene *mat1-2-1* (116).

Mycotrophy

Mycoparasitism, wherein a fungus derives nutrients living in association with another fungus, is an ancestral trait of *Trichoderma* (67). Mycoparasitic *Trichoderma* species can destroy the host, but there are biotrophic mycoparasites that do not kill the host (for discussion, see Reference 29). Druzhinina et al. (36) broadened the concept of mycoparasitism to other nutritional relationships between fungi and defined mycotrophy to include the ability of *Trichoderma* to feed on dead fungi as well. Genome analysis of two mycoparasitic species illustrated the richness of genes encoding compounds (e.g., antibiotics, CWDEs, and those for self-defense) required to kill other fungi (67). Furthermore, *Trichoderma* can also kill plant-parasitic nematodes, expanding their range of biocontrol (19).

The mycoparasitic interaction is apparently programmed by host signals (138), resulting in infective structures and enzyme secretion. Prior to physical contact, mycoparasitism begins with recognition of the prey, using diffusible signals such as oligochitins (27). Upon contact, the mycoparasite attaches to the host (probably mediated by hydrophobin-like proteins) followed by the formation of papillae/appressoria-like structures and mycoparasitic coiling. Hyphal coiling may be part of a more general response to a filamentous substrate, as *Trichoderma* coils upon interaction with lectin-coated nylon fibers (58). The production of chitinases, glucanases, and proteases facilitates the flow of nutrients into the mycoparasite and of degradation of the host. *Trichoderma* parasitizes not only active hyphae but also resting structures/propagules, such as sclerotia and perithecia (59, 95). Mycoparasitism-related genes respond transcriptionally to the prey, and several studies identified genes expressed during interactions of *Trichoderma* species with plant pathogens (1, 13, 140). Despite multiple efforts, no significant advances have been made in the determination of the factors directly involved in regulation of mycoparasitism-induced genes. Signal transduction pathways involved in mycoparasitism have been elucidated to some extent (10, 65, 101, 103). The mitogen-activated protein kinase (MAPK) TmkA/Tvk1 (ortholog of the pathogenicity-related MAPKs of phytopathogens) is not essential for mycoparasitism of *T. virens* on *Rhizoctonia solani*, and the production of CWDE is actually increased by the lack of Tvk1 (83). This pathway is crucial for antagonism of *S. rolfsii*, for which parasitism of sclerotia is important (94). TmkB had similar influence on antagonism against *S. rolfsii*, indicating overlapping functions of these two MAPKs (72).

Symbiosis and Endophytism

The saprophytic fitness of *Trichoderma* species has enabled their establishment in soil and rhizosphere and often within roots where hyphae grow between cortical cells (145).

Trichoderma-root interactions involve recognition, attachment, penetration, colonization, and nutrient transfer from the root (90). Two hydrophobin-like proteins, TasHyd1 from *T. asperelloides* and Qid74 from *T. harzianum*, are involved in attachment of *Trichoderma* to roots. The expansin-like protein swollenin (8) and an endopolygalacturonase (87) contribute to root colonization. *T. virens* was shown to produce auxins and related compounds that may facilitate root colonization by enhancing root growth (25). The transport of sucrose from plants with subsequent intracellular hydrolysis by *T. virens* has been shown. This source-sink communication may be central to the mutualistic interaction, influencing proliferation of *Trichoderma* in the rhizosphere and root (133, 134). Although well known for their ability to colonize the rhizosphere with limited root penetration, some *Trichoderma* species are known to reside in plants as typical endophytes, entering through trichomes by producing appressoria-like structures (2).

Penetration into the plant would initially imply activation of plant immunity. Symbionts, however, circumvent or remodel the plant immune response (146). Lorito et al. (78) presented a model for *Trichoderma*-plant interactions, superimposing the predicted phases on the zigzag model of Jones & Dangl (61). Studies of the plant's response must take into account the time frame of infection and defense: *Trichoderma* may establish the symbiosis in a time window when plant defenses are low (88), which could occur during the pathogen-associated molecular pattern (PAMP)-triggered or effector-triggered sensitivity stages (61).

Whether the *Trichoderma*-induced defense response is typical of the ISR, which is induced by plant growth–promoting rhizobacteria, or of the SAR, which is induced by necrotrophs, is debatable. The defense pathways triggered by *T. asperellum* and *Pseudomonas fluorescens* were found to be highly similar. The root-specific transcription factor MYB72 appears to function as an early node of convergence in the signaling pathways triggered by these two groups of beneficial root microbes. Additionally, *T. asperellum*–induced ISR was fully functional in an *Arabidopsis* salicylic acid (SA)-impaired mutant, *sid2*, indicating that the jasmonic acid (JA)/ethylene (ET) pathway, and not the SA pathway, is involved (114). *Trichoderma*-induced defense response has been traditionally treated as ISR (126). ISR, SAR, or both could be activated depending on the timing of interactions and the applied inoculum load of *Trichoderma* (113). Application of *T. atroviride* to *Arabidopsis* roots induced an overlapping expression of genes related to SA and JA/ET pathways, along with the genes responsible for the synthesis of the antimicrobial phytoalexin camelexin (24). Infection of roots by *T. asperellum* did not provoke major transcriptomic changes in *Arabidopsis* leaves, but genes for pathogenesis-related (PR) proteins were upregulated upon challenge inoculation by the pathogen *Pseudomonas syringae* (9). Interestingly, the transcription factor WRKY40, which is known to contribute to *Arabidopsis* susceptibility to bacterial infection, was downregulated. Except perhaps for the role of a MAPK pathway in *T. virens* (137) and a MAPK in cucumber (125), the molecular details of the signal transduction pathways in the plant or fungus have not been studied in detail. Signaling by the plant in response to *Trichoderma* may prove to be a variation of the known interaction with other beneficial microbes (146).

Trichoderma species produce several proteinaceous elicitors, including a xylanase, peptaibols, and the small cysteine-rich secreted protein Sm1/Epl1, that can trigger defense responses in plants when produced in planta or applied in pure form (34, 121). The xylanase acts as a microbe-associated molecular pattern (MAMP), which is detected by an extracellular leucine-rich repeat (LRR) receptor (104). The ceratoplatanin (CP)-related Sm1 is the best characterized of the small cysteine-rich secreted protein elicitors. Only the monomeric form is assumed to be active, and the deletion of the corresponding gene results in a loss of ISR response in maize (35).

Some fungal and oomycete effectors have a signature RXXL-DEER motif for transport

Necrotroph: parasite or pathogen that kills the host first and then feeds on it

Elicitor: molecule that induces host defense; effector usually refers to pathogen factors with virulence targets in the plant

Peptaibols: small peptides of non-ribosomal origin that have a high content of α-iso aminobutyric acid and C-terminal alcohol

into plant cells (62), which is not obvious in Sm1. Understanding the structural differences between *Trichoderma* and pathogen CP family elicitors might be insightful for solving the mystery of how *Trichoderma* Sm1 is not phytotoxic and instead induces resistance. The specificity might reside in the plant receptors or fungal elicitors [or in the timing (as mentioned above)] when the elicitor/effectors are presented to the plant immune system.

Gene cluster: a group of functionally related genes present in tandem in the genome

Transcriptome: the complete set of mRNA species present at a given time; snapshot of gene expression

Secondary Metabolism

Even though *Trichoderma* species have been known for decades to produce numerous secondary metabolites [peptaibols, epipolythiodioxopiperazines (ETPs), volatile and nonvolatile terpenes, pyrones, polyketides, and siderophores] (31), much remains to be understood regarding their ecological roles and the genetics of their biosynthesis. Pioneering work identified the *tex1* gene (144) and established its role in the biosynthesis of the 18-residue peptaibol trichovirin II. The transcription factor Thctf1 regulates the biosynthesis of the volatile antifungal compound 6-pentyl-2H-pyran-2-one (6-PP) (106). A trichothecene-like (TRI) cluster has been identified in *Trichoderma brevicompactum*, producing the phytotoxin trichodermin (12). *Trichoderma arundinaceum*, in contrast, is beneficial to plants and produces harzianum A, a growth-promoting trichothecene (79). This is an example in which the same gene cluster in different species has been fine-tuned to the ecological needs of the producing strain. In contrast, gliotoxin is produced by two phylogenetically distinct organisms with diverse ecological niches. The same compound is used for pathogenesis of *Aspergillus fumigatus* in humans and for suppression of plant pathogens in *T. virens* (P.K. Mukherjee, W.A. Vargas, C.M. Kenerley, unpublished results). Deletion of the 4-PPTase in *T. virens* resulted in the production of nonpigmented conidia and nonproduction of nonribosomal peptides (NRPs), illustrating its involvement in biosynthesis of polyketides and NRPs (135). Consequently, the deletion mutant failed to induce resistance in *Arabidopsis* and to inhibit pathogenic fungi through the production of antibiotics.

Secondary metabolism is a tightly regulated process in fungi and often linked to reproduction. The VelB-LaeA-VeA complex appears to be a universal regulator of secondary metabolism in fungi (4). The *T. virens veA* ortholog *vel1* is involved in regulation of gliotoxin biosynthesis and many other secondary metabolism-related genes, including polyketide synthases (PKSs), NRP synthetases (NRPSs), and methyl transferases, as well as conidiation, hydrophobicity, mycoparasitism, and biocontrol. The *T. reesei* ortholog (LAE1) of the *Aspergillus* protein methyltransferase LaeA regulates genes for lignocellulose degradation and secondary metabolism, among others (63, 119).

GENOMES AND GENOMICS

The first member of the genus sequenced was *T. reesei/H. jecorina* (80). This species is an unusually good producer of cellulases but a limited biocontrol agent. When the sequences of the two biocontrol species *T. atroviride* and *T. virens* were assembled, annotated, and analyzed, the appearance of many unique genes and expanded families were realized that might explain the ability of these mycoparasites to attack other fungi and interact with plants (67). In the past year, the genomes of *T. harzianum* and *T. asperellum*, which are biocontrol species that belong to other phylogenetic branches (for detailed phylogeny, see Reference 67), have become available (**Figure 3**). Equally important for human welfare is the completion of the whole-genome sequencing of *T. citrinoviride* and *T. longibrachiatum*, two species known to cause human health problems. This will facilitate comparative transcriptome analysis among these species, making it possible to assess the microevolutionary events occurring on a relatively recent timescale and exposing new genes whose products have promoted invasion of new niches.

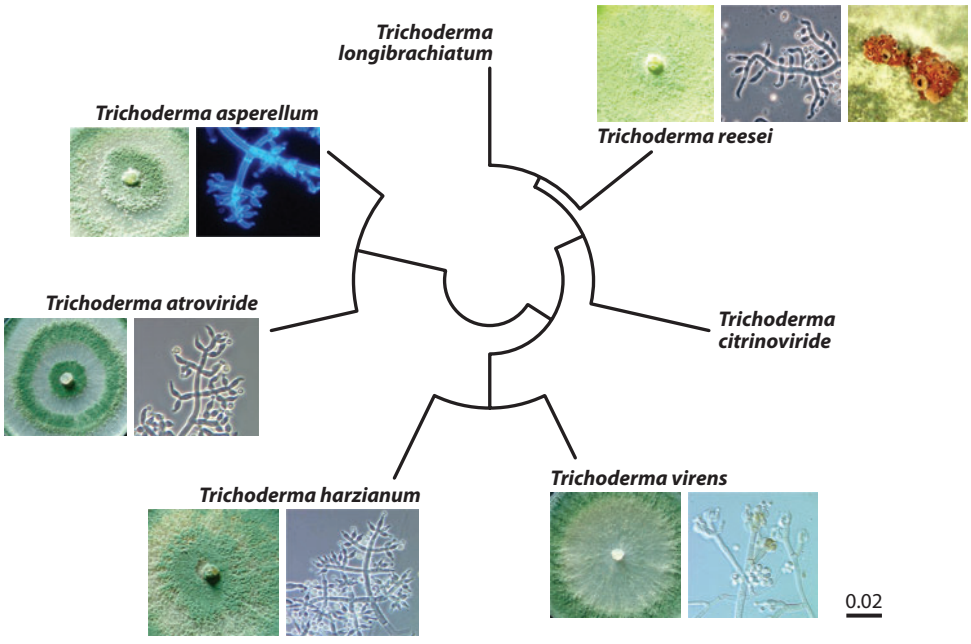


Figure 3

Evolutionary relationships of the seven sequenced *Trichoderma* species. The evolutionary history was inferred using the minimum evolution method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA 4. Pictures are in part taken from the *Trichoderma* online database (107).

Key Features of the Genomes

The analysis of the first three genomes (*T. reesei*, *T. virens*, and *T. atroviride*) has enriched our knowledge of the evolution of mycoparasitism in the context of ecological fitness of *Trichoderma* spp. (36, 67). The mycotrophic *T. atroviride* and *T. virens* have, overall, a larger predicted gene model set and expanded families for CWDEs (in particular chitinases), secondary metabolites, and small secreted proteins than the primarily saprophytic *T. reesei*; a comparison of this trend to those families revealed in the more recently sequenced species should prove to be interesting. **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) presents key features of the seven genomes that have been sequenced to date.

High-Throughput Analyses: The Genome-Wide View of Morphogenesis and Biocontrol

The availability of *T. reesei*, *T. virens*, and *T. atroviride* expressed sequence tag (EST) libraries and genomes has enabled the examination of gene function in a holistic manner instead of via isolated studies. Research prior to 2010 was based on cDNA microarrays and a few proteomic studies (for a detailed summary, see Reference 78). With the release of the genomes, oligonucleotide arrays were designed and now are already superseded by RNA-Seq, which has the advantages of greater depth and new bioinformatic tools as well as the potential to follow transcript abundance in sequenced plant partners, such as *Arabidopsis*, tomato, and maize, and in fungal hosts, such as *N. crassa*.

Supplemental Material

Sexual and asexual development. Availability of whole-genome sequences has greatly facilitated research toward a detailed understanding of development in *Trichoderma*. Investigation of the transcriptomes of strains able to undergo sexual development under certain conditions versus those that do not reproduce sexually under the same conditions revealed downregulation of three conidiation specific genes, the h-type pheromone *hpp1*, and several genes involved in propheromone processing and secretion. Further analysis has indicated that HPP1 may have a role in promoting conidiation under constant illumination. In contrast, cellulase and hemicellulase genes were upregulated along with other genes required for vegetative growth under conditions favoring sexual development (18). Apparently, abundant photoconidiation as initiated by light counteracts sexual development, with ENV1 playing an important role in keeping the balance between these developmental stages.

The availability of the genome sequence also permitted genome-wide analysis of gene expression by high-throughput sequencing under conditions that trigger conidiation. This analysis allowed the identification of 331 white light–regulated genes and 204 genes responsive specifically to blue light, which induces conidiation (M. García-Esquivel, U. Esquivel-Naranjo, M. Hernandez-Onate, A. Herrera-Estrella, unpublished results). The set of genes responsive to light is enriched in the functional categories of metabolism, stress, cellular transport, cell cycle and DNA processing, transcription, and cell differentiation. Additionally, the genome-wide transcriptome analysis demonstrated that at least ten transcription factors are regulated by light, suggesting that the whole process involves a cascade of transcriptional events (14; M. García-Esquivel, U. Esquivel-Naranjo, M. Hernandez-Onate, A. Herrera-Estrella, unpublished results). These transcriptomic analyses further revealed that components of the oxidative stress pathway were responsive to light. A role of ROS in conidiation induced by light has been proposed (M. García-Esquivel, U. Esquivel-Naranjo, M. Hernandez-Onate, A. Herrera-Estrella, unpublished results). Similarly, a recent transcriptome analysis using high-throughput sequencing indicated that 933 genes are transiently regulated in response to injury, which also leads to conidiation. Consistent with what was observed in response to light, a significant number of injury-responsive genes encode proteins related to oxidative stress. These observations led to the suggestion of the involvement of NADPH oxidases (Nox) in the process. Gene replacement of the two catalytic subunits of Nox confirmed that Nox1 is indeed a determinant for injury-induced conidiation (54). The availability of these data in a temporal frame allows the identification of genes involved in the morphogenetic transition independently of the cue triggering the process.

Genome-wide transcriptome analysis of conidiating cultures of *T. reesei* revealed a considerable increase in the abundance of CAZyme-encoding genes, especially for cellulases (84). This is in accordance with the occurrence of cellulolytic enzymes on the spore surface of *T. reesei* (69). In total, 900 genes belonging to the functional categories of carbohydrate metabolism, amino acid metabolism, and lipid metabolism (as well as transcription factors, signaling components, proteases, and stress response proteins) were found to be regulated during conidiation (84).

Hydrophobins were among the first developmental markers discovered (*T. harzianum* Srh1) (97). Members of this class of small secreted proteins with a conserved signature of eight cysteines determine the physical properties of the surface. Unique to fungi, hydrophobins can self-assemble into a hydrophobic rodlet layer, although this does not seem to occur with *T. harzianum* Srh1. The BLR-1/BLR-2 photoreceptors are responsible for light regulation of *T. atroviride* hydrophobins (85). The G-α subunit GNA1 of *T. reesei* and Vel1 of *T. virens* regulate hydrophobin genes (93, 115). *Trichoderma* genomes encode an unusually large number of hydrophobins (66), perhaps providing the flexibility in surface

properties needed for conidiation, mycoparasitism, and interaction with plant roots.

Mycoparasitism. Postgenome experiments have been conducted to examine mycoparasitism on a genomic scale. Chitinases, which are considered highly important in mycoparasitism, have been the main focus. A comparative analysis of chitinases revealed that *Trichoderma* genomes harbor between 20 and 36 different genes that encode chitinases, with chi18-13 and chi18-17 significantly expanded in the mycoparasites *T. atroviride* and *T. virens* (57). Chitinases of subgroup C (expanded in mycoparasitic species) were induced in *T. atroviride* in response to *Botrytis cinerea* but not to *R. solani* (47). Genomic and transcriptomic analyses showed that the genes neighboring these chitinases in *T. atroviride* encoded proteins composed solely of LysM modules and were coregulated with the chitinases. Interestingly, the expression pattern of these chitinases was different in *T. virens* and governed by a combination of colony-internal and colony-external signals (54). Complex expression patterns in different parts of the fungal colony are indicative of additional roles of these chitinases in cell wall reprogramming in *T. virens* (45). In a recent analysis of chitinase expression in *T. atroviride* and *T. virens*, chitinases were found to have overlapping functions in self and nonself (host) cell wall degradation. Also, cell wall degradation is regulated by substrate accessibility (protected in self versus unprotected in host) (46). An EST-based analysis of gene expression patterns at the beginning of physical contact between *T. atroviride* and *B. cinerea/R. solani* identified 66 genes that were overexpressed during the onset of mycoparasitism (123). The most abundant genes were involved in posttranslational processing and amino acid metabolism. Using a high-throughput transcriptome analysis, 175 genes from *T. atroviride* were identified that responded to the presence of *R. solani*. Strongly upregulated genes include *swo1* and *axe1* as well as genes encoding aspartyl protease PapA and a trypsin-like protease Pra1 (102).

Plant interactions. The availability of *Trichoderma* genomes coupled with those of several plants permits the bidirectional interactions at the genome scale to be studied. Analysis of transcriptome changes in *T. harzianum*, *T. virens*, and *Trichoderma hamatum* during interactions with tomato plants revealed that 1,077 genes were regulated when the fungi were grown with tomato (593 in *T. harzianum*, 336 in *T. virens*, and 94 in *T. hamatum*, six genes being common to all three). Interestingly, genes encoding enzymes needed for chitin degradation (N-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, and chitinase) were significantly overexpressed in all three *Trichoderma* spp. during early interactions with tomato plants (105). From the plant side, studies on the transcriptomic response of *Arabidopsis* to *T. harzianum* inoculation revealed that after 24 h of incubation SA- and JA-related genes were downregulated, whereas several genes related to abiotic stress response were upregulated (88). Contrary to this, during the first three hours of interaction between cucumber roots and *T. asperellum*, SA and JA levels and peroxidase activity increased in the cotyledons, depending on the inoculum levels of the fungus (113). At high inoculum levels, proteomics showed that proteins involved in ROS scavenging, stress response, isoprenoid and ET biosynthesis, photosynthesis, photorespiration, and carbohydrate metabolism were differentially regulated in the cotyledons by *T. asperellum*. Additionally, there is a close resemblance between ISR-prime and SAR in *Arabidopsis* plants in response to *T. hamatum* inoculation (82).

Secreted proteins are likely to play a role in the communication between *Trichoderma* and plants, just as they do in plant-pathogen and plant-mycorrhizae interactions. The *Trichoderma* genomes have now been shown to contain a large repertoire of small cysteine-rich secreted protein-like genes (67), potentially encoding hundreds of possible elicitors. The challenge is to identify which are relevant and then test the proteins for activity (11). Initial bioinformatic classification cannot yet provide

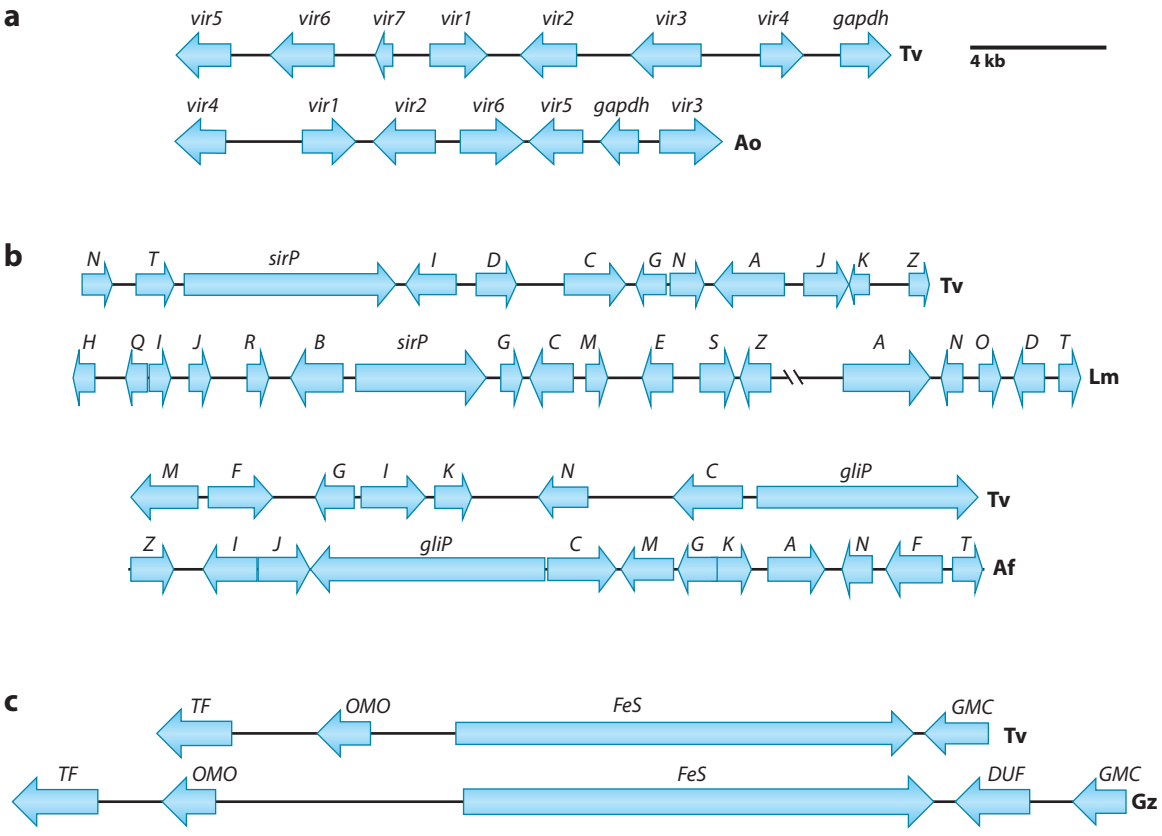


Figure 4

Examples of secondary metabolism–related gene clusters in *Trichoderma virens* (Tv). (*a*) The *vir* cluster in Tv and in *Aspergillus oryzae* (Ao). (*b*)The SirP/GliP cluster in Tv, *Leptosphaera maculans* (Lm), and *Aspergillus fumigatus* (Af). (For detailed annotations, please see Reference 92). (*c*) The ferricrocin siderophore gene cluster in Tv and *Gibberella zeae* (Gz). Abbreviations: DUF, DUF domain protein; FeS, ferricrocin synthetase; GMC, GMC oxidoreductase; OMO, ornithine monooxygenase; TF, transcription factor.

a firm indication of which proteins are likely elicitors, but it can at least guide future efforts.

Secondary metabolism. A thorough understanding of the processes involved in production of secondary metabolites is important for effective, harmless utilization of *Trichoderma* spp. Similar to other fungal genomes, most of the secondary metabolism–related genes in *Trichoderma* reside in clusters, with many acquired by horizontal gene transfer, and the majority appear to be silent under standard laboratory conditions (for examples, see **Figures 4** and **5** and **Supplemental Figure 1**) (3, 91, 92, 96). *Trichoderma* secondary metabolites may be grouped into peptaibols, small NRPs (e.g., gliotoxin, siderophores), polyketides, terpenes, or pyrones. An extensive survey of *Trichoderma* genomes revealed the presence of three types of peptaibol synthetases: the long (18–20 modules), medium (14 modules), and short (7 modules) (30, 96). One of the most interesting findings driven by genomics was the ability of Tex2, the 14-module NRPS, to synthesize 11- and 14-residue peptaibols in *T. virens* (102), later confirmed in *T. reesei* (38). On the basis of sequence alignment and the absence of an 11-module peptaibol synthase in either of the genomes, module skipping is suggested to be the mechanism of synthesis of these two classes

Supplemental Material

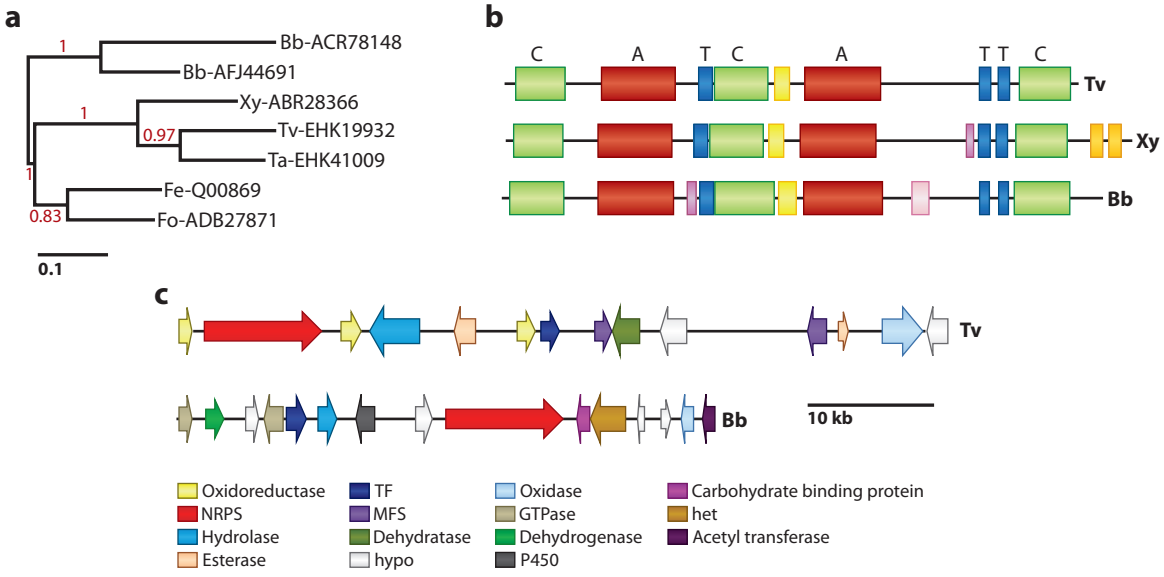


Figure 5

Organization of Tex15 gene cluster and its relatedness to orthologous nonribosomal peptide synthetases (NRPSs). (*a*) Relatedness of the first "A" domain of Tex15 of *Trichoderma virens* (Tv), *Trichoderma atroviride* (Ta), *Beauveria bassiana* (Bb) (beauvericin synthetase and bassianolide synthetase), *Xylaria* sp. (Xy) (bassianolide synthetase), *Fusarium equisetti* (Fe) (enniatin synthetase), and *Fusarium oxysporum* (Fo) cyclic peptide synthetase. (*b*) The domain organization of Tv-Tex15, Bb (bassianolide synthetase), and Xy (bassianolide synthetase). (*c*) Putative gene cluster of Tv-Tex15 and Bb-bassianolide synthetase. Note that this gene cluster is absent in *Trichoderma reesei*. Abbreviations: A, adenylation; C, condensation; MFS, major facilitator superfamily; T, thiolation; TF, transcription factor.

of peptaibols by one NRPS. The *T. virens* Tex2 was shown to synthesize a total of 88 peptaibols belonging to 11- and 14-residue groups. This is an example how a single gene may generate many structurally different compounds that have antimicrobial properties and play a role in the interactions with plants and other animals. The ETP class of secondary metabolites is important for their role in plant and human pathogenicity and biocontrol (92). A group of *T. virens* strains designated as Q strains produce gliotoxin, which is a virulence factor in *A. fumigatus*. In *A. fumigatus* and *T. virens*, gliotoxin has been shown to be produced by a gene cluster with the NRPS GliP as the core enzyme (**Figure 4*b***) and regulated by LaeA/VeA (92). The members of this cluster are coregulated during mycoparasitism (1, 92). Part of the cluster is present in *T. reesei* but is not regulated during confrontation with other fungi nor is gliotoxin produced. Analysis of the function of gliotoxin by gene knockout in *T. virens* has demonstrated a role in biocontrol of *Pythium ultimum*, degradation of sclerotia of *S. sclerotiorum*, and mycelial growth on agar (P.K. Mukherjee, W.A. Vargas, C.M. Kenerley, unpublished results). A phylogenomic analysis of the evolution of this cluster suggests that the gliotoxin gene cluster was horizontally transferred from *Aspergillus* spp. to a common ancestor of *T. virens* and *T. reesei*. Although mycoparasitic *T. virens* retained the functional cluster, *T. reesei* lost part of it during evolution.

Iron is an essential element for survival, and siderophores are important for the acquisition of iron in a competitive environment. On average, *Trichoderma* spp. produced 12–14 siderophores (74). The genomes of *T. virens* and *T. reesei* contain a gene for extracellular siderophore production (SidD ortholog) in addition to the functional NPS6 cluster that is conserved across the three species (92). Gene function analysis indicated that SidD is not involved in the biosynthesis of siderophores (P.K.

Phylogenomics: research at the intersection between genome data and evolutionary analyses

Mukherjee, R. Schuhmacher, C.M. Kenerley, unpublished results). The wild-type strain produced 12 extracellular siderophores of both fusarinine and coprogen class and included six unknown compounds. Deletion of NPS6 eliminated 10 of these 12 siderophores. Similarly, all three *Trichoderma* spp. maintain a conserved gene cluster for the biosynthesis of the intracellular siderophore ferricrocin (**Figure 4*c***).

Supplemental Material

PKS/NRPS hybrid clusters (see **Supplemental Figure 1**) produce many interesting secondary metabolites in fungi (e.g., lovastatin, equisetin, fusarin, cytochalasan, and tenellin). Mukherjee et al. (91) identified a PKS/NRPS hybrid that is involved in the ISR response in maize. This gene cluster contains two PKS/NRPSs, much like the Ace1 cluster of Mg that acts as an avirulence factor in rice genotypes that contain the corresponding R gene (23). Baker et al. (3) presented a detailed phylogenomic analysis of PKSs in *Trichoderma*, but functional studies are still to be done.

Trichoderma spp. also produce volatile and nonvolatile terpenoids (like viridin and viridiol produced by *T. virens*). Prior to the release of the genome of *T. virens*, Mukherjee et al. (89) identified a partial gene cluster from *T. virens* and suggested its involvement in the biosynthesis of viridin. Recent genetic studies revealed that this cluster, which again appears to be acquired by *T. virens* from *Aspergillus oryzae*, is responsible for the biosynthesis of a novel class of volatile sesquiterpenes (**Figure 4*a***). These compounds are not produced by *T. atroviride* and *T. reesei*, which correlates with the absence of this cluster in these two species (28). Strains of *T. atroviride* produce the volatile compound 6-PP, which has beneficial effects on plants and is antimicrobial. A lipooxygenase gene unique to *T. atroviride* (Triat1:33350) has been suggested to be involved in biosynthesis of this compound (67). *Trichoderma* spp. also produce trichothecenes, such as trichodermin and harzianum A, via a TRI cluster that is significantly different from that of *Fusarium* TRI clusters (12, 79). Deletion of Tri4, a Cyt P450 monooxygenase resulted in reduced antifungal activity of *T. arundinaceum* against *B. cinerea* and *R. solani* and also reduced induced resistance in tomato (79).

TRICHODERMA RESEARCH IN THE POSTGENOME ERA: UNLIMITED OPPORTUNITIES

High-Throughput Transcriptomes and Functional Analysis

One of the greatest challenges in postgenome research is to elucidate the functions of every gene in a short span of time. The availability of an almost complete knockout library for *N. crassa* has accelerated research with this model fungus (99). Similar efforts are underway for *Magnaporthe oryzae* (20). In a medium-scale approach, mutants in 657 transcription factor genes were used to construct a phenotype database for *Fusarium graminearum* (127). The foundations for high-throughput functional genomics have been established for *T. reesei* with a procedure for efficient construction of knockout mutants (111). However, a dedicated community is required to resolve the impact of the most interesting groups of genes in a coordinated endeavor. Additionally, contributions and support of industrial partners working with different *Trichoderma* spp. can greatly influence the speed with which knockout libraries can grow. As annotation of *Trichoderma* genomes is becoming more and more refined, gene groups can be selected according to their relevance to agriculture, industry, and medicine. Although the focus should first be on genes with striking regulation under certain conditions of interest, closely related genes of the same group should not be neglected, considering the largely unknown role of posttranslational modification and regulation in *Trichoderma*. The next logical step is to obtain a whole-genome collection of tagged genes, which will enable determination of the intracellular location of proteins in vivo and coimmunoprecipitation with antibodies to the tags to identify protein interactions. The advent of technology for large-scale transcriptome analysis (microarrays and RNA-Seq) created opportunities to identify strong constitutive promoters as well as inducible

promoters. Traditionally used universal promoters for driving the expression of desirable genes as well as for the silencing of the undesirable ones will be replaced with novel ones specifically chosen for expression, for example, in interaction with roots, during production of spores, or in the light. Advancements in high-throughput next-generation sequencing (NGS) have made it possible to identify mutations in classical mutants of *T. reesei* (73, 118). The combination of classical mutagenesis and NGS will be an invaluable approach to identify novel genes with interesting functions, particularly as some such mutants are already available and more could easily be generated (89).

Comparative Genomics and Identification of Genetic Markers

There are more than 200 well-defined species in the genus *Trichoderma*. A comparative genomics/transcriptomics analysis as applied for *N. crassa* (37) would be required to demonstrate the genetic basis that differentiates these species/strains. Such an endeavor would assist in the discovery of traits associated with beneficial or harmful species/strains. Once identified, trait-specific genetic markers can be developed for rapid and early screening of strains. Hence, the need for screening of thousands of strains of *Trichoderma* in vivo could be optimized for known targets, shortening the time for development of commercial strains (42). Several such efforts have been performed recently to elucidate the molecular basis for high-performance cellulase-producing strains of *T. reesei* (73, 139).

Genome Mining for Useful Genes and Gene Products

Conidiation is an extremely important process for survival and development of biocontrol formulations. The process is well understood in *Aspergillus nidulans* with several genes (e.g., *brlA*, *stuA*, *medA*, *abaA*, *wetA*, *flbB*, *flbC*, *flbD*, and *flbE*) required for the normal production of conidia. The transcriptional regulators encoded by *brlA*, *stuA*, *medA*, *abaA*, and *wetA* program sequential development from conidiophore differentiation to spore maturation (38). Mining of the *Trichoderma* genomes in a search for orthologs of these genes showed that almost all genes are present (14). Interestingly, there is no ortholog of *brlA*, which determines the conidiophore vesicle, a structure apparently not formed in *Trichoderma* conidiophores (22). These findings could have an impact in the production of conidia-based formulations. As discussed previously, many of the secondary metabolism-related gene clusters are silent under standard laboratory cultivation conditions, but may be selectively woken by modern genetic tools (75). As an example, the Tex15 cluster depicted in **Figure 5** (92) contains an NRPS that is an ortholog of and structurally similar to the synthetases for the insecticidal metabolites bassianolide and beauvericin. Both of these genes are part of "not-so-similar" gene clusters in *T. virens/T. atroviride* and *B. bassiana*, and would be expected to catalyze the formation of a compound that is similar to beauvericin and bassianolide. Both clusters contain a GAL4-type transcription factor. Theoretically, this cluster could be activated, which would lead to the detection of novel metabolites. Similarly, all six PKS-NRPS gene clusters from the three *Trichoderma* spp. have at least one transcription factor, suggesting that these clusters can be activated to exploit the biosynthesis of unique compounds.

Plant biotechnology will also benefit from *Trichoderma* genome mining. The most successful examples of transgenic research and commercial applications are the expression of microbial genes in plants (e.g., Cry toxins from *Bacillus thuringiensis* and herbicide-resistant genes from *Streptomyces* sp.) rather than the exploitation of plant genes. Even the most common selectable markers are of microbial origin (such as *npt* and *hph*). Microbial genes seem to suffer less from gene silencing compared with plant genes expressed in plants, and codon optimization is not normally required. The successful expression in plants of an endochitinase gene from *T. harzianum* that imparted tolerance to many fungal (both foliar and root) infections (77) encouraged further attempts

Genome mining: extracting useful information from the genome of an organism, mostly by using bioinformatics approaches

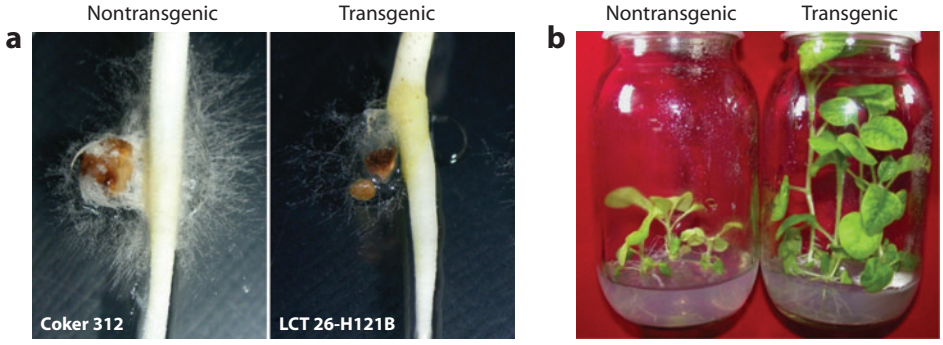


Figure 6

Expression of *Trichoderma* genes in transgenic plants imparts tolerance to biotic and abiotic stresses. (*a*) Tolerance of cotton roots expressing an endochitinase from *Trichoderma virens*. Please note that *Rhizoctonia solani* infects roots in nontransgenic plants (*left*), whereas it shows a lower degree of infection in transgenic roots. Photo courtesy of Keerthi Rathore. (*b*) Tolerance of tobacco plants expressing a *T. virens* glutathione S-transferase gene to 100 μM cadmium chloride. Photo courtesy Prachy Dixit.

to express genes from *Trichoderma* in plants to promote tolerance to biotic and abiotic stresses (32, 33, 53) (**Figure 6**). The *Trichoderma* genomes contain several hundred genes that are candidates as transgenes, such as the numerous chitinases and glucanases, lectins (some lectins have insecticidal properties and are an active field of research), cytochrome P450s and glutathione S-transferases (GSTs). Most *Trichoderma* strains are naturally resistant to many xenobiotics, and one goal would be to identify the genes or pathways involved in the degradation of these compounds and engineer them into plants. Similarly, metal transporters can be transferred to plants to aid in bioremediation. Further research would identify candidate genes for transferring to plants for tolerance to biotic and abiotic stresses, such as cold, drought, salinity, pH, and herbicide resistance.

CONCLUSIONS

Research with *Trichoderma* spp. has accelerated considerably with the availability of the fully sequenced genomes. At the same time our understanding of the lifestyles of different species of the genus is now supported by information on their genetic inventory, which determines their capabilities and limitations. Discrepancies and surprises from decades of research now appear in a new light. The newly acquired genome sequences have also provided impetus for new research. For instance, the production of so many small secreted proteins, the expanded families of CWDEs, and putative secondary metabolite biosynthetic enzymes are new areas that require cooperative efforts from genetic, chemical, and physiological approaches. The availability of numerous *Trichoderma* genome sequences would facilitate interchange of research among the different species of the genus. Regulatory patterns, for example, cellulase transcription factors and signaling components in *T. reesei*, can be compared with the response of effective biocontrol species to plant material or other fungi. Such comparative studies will provide intriguing new insights into the physiology of *Trichoderma* species with different lifestyles, e.g. mycoparasites and efficient plant cell wall degraders. Application of high-throughput genome, transcriptome, and metabolome analysis of native and mutant strains will result in the identification of novel genes with specialized functions that are relevant to biomass degradation, biocontrol, and human pathogenicity. Nevertheless, future efforts will be necessary to integrate these molecular maps of physiological processes; new questions will arise and ultimately pry us away from the computer to the bench to evaluate a multitude of hypotheses.

Bioremediation: managed process in which a microbiological catalyst is applied to alleviate contaminants in the environment (phytoremediation if plants are employed)

SUMMARY POINTS

1. In recent years, the genomes of seven *Trichoderma* species have been sequenced; three have been analyzed and four are in progress. New genome sequences will enable the comparison of phylogenetically diverse species as well as mycoparasitic and human pathogens.
2. Comparative genome analysis has indicated the evolution of *Trichoderma* as mycoparasites. More high-throughput experimentation is needed to fully understand the biology of these economically important fungi.
3. The future of *Trichoderma* research is likely to be balanced between basic studies for unraveling genetic secrets of biological control and application-oriented work to mine the genome for useful genes and gene products.
4. Achievement of sexual crossing of *T. reesei* under laboratory conditions highlights new avenues for research in sexual development within *Trichoderma* and will greatly facilitate strain improvement for biocontrol.
5. *Trichoderma* genomes are vast reservoirs of many cryptic secondary metabolism–related genes, of which the biosynthetic products of only a few are known. Genetic intervention will revolutionize this field of research, with many novel metabolites uncovered.
6. Asexual development is important for survival and biocontrol formulation. Genomics and transcriptomics have begun to yield interesting information regarding light- and injury-induced conidiation.
7. The mycoparasitic *Trichoderma* species harbor, on average, 200 genes for elicitor-like proteins, although the function of only one has been studied. The elucidation of which of these proteins are involved in *Trichoderma*-plant and *Trichoderma*-fungus interactions presents a challenge, but one that may yield important gains for agriculture.
8. *Trichoderma* genomes are a source of candidate genes for developing transgenic plants that are tolerant to biotic and abiotic stresses. Development of engineered plants will be benefited by *Trichoderma* genome mining.

FUTURE ISSUES

1. To explore the phylogenetic diversity of this genus and reveal novel niche-related genes, additional beneficial (such as endophytes) and harmful (such as aggressive colonizers of mushrooms) *Trichoderma* strains should be sequenced and combined with in-depth expression analysis. This research should be complemented by functional genomics studies to evaluate hypotheses formed from genome analysis.
2. The process of induction and regulation of enzyme expression at the molecular level should be investigated. Additionally, the environmental cues along with the transmission of the respective signal to the promoters of the output pathways should be considered. Such knowledge of the physiology will further improve enzyme production in the microbial cell factory *T. reesei* and enhance the mycoparasitic potential of biocontrol strains. One might be able to develop *Trichoderma* strains with dual applications in biocontrol or enzyme production (e.g., a strain of *T. reesei* with improved biocontrol abilities).

3. Efforts should be made to extend the scope of *Trichoderma* research beyond agriculture to biomedical applications with high-throughput screening of peptaibols and other known and yet to be discovered (genomics-driven) metabolites.
4. A comparison between the genomes of saprophytic and human pathogenic strains has the potential to unravel what genes may be involved in human pathology. Comparing *T. reesei* with *T. longibrachiatum* (phylogenetically closely related) may provide evidence of which genes were acquired or modified by *T. longibrachiatum* to acquire pathogenicity.
5. The initiation of a detailed investigation of sexual development and its determinants in *T. reesei* will provide a blueprint for other *Trichoderma* species.
6. The genetic basis of chlamydospore production in *Trichoderma* should be investigated. This information may provide a rationale for the abundance of chlamydospore production in some species but the rarity of production in other species.
7. The identification of the plant receptors for *Trichoderma* elicitor proteins and effectors that may reprogram a host's genetic machinery can serve as the foundation for understanding how these symbionts avoid or reduce the host defense reaction.
8. The ecological impact of large-scale applications of a single fungal species for biocontrol should be evaluated to establish a knowledge base for the safe use of *Trichoderma*. Similar experiments should be conducted for abundant metabolites produced by these fungi. With the current knowledge of the opportunism of *Trichoderma* and the human pathogenicity of some isolates, an investigation of the potential health risk for individuals manufacturing biocontrol fungi would seem prudent.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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