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MINI-REVIEW

Biology and biotechnology of *Trichoderma*

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Abstract Fungi of the genus *Trichoderma* are soilborne, green-spored ascomycetes that can be found all over the world. They have been studied with respect to various characteristics and applications and are known as successful colonizers of their habitats, efficiently fighting their competitors. Once established, they launch their potent degradative machinery for decomposition of the often heterogeneous substrate at hand. Therefore, distribution and phylogeny, defense mechanisms, beneficial as well as deleterious interaction with hosts, enzyme production and secretion, sexual development, and response to environmental conditions such as nutrients and light have been studied in great detail with many species of this genus, thus rendering *Trichoderma* one of the best studied fungi with the genome of three species currently available. Efficient biocontrol strains of the genus are being developed as promising biological fungicides, and their weaponry for this function also includes secondary metabolites with potential applications as novel antibiotics. The cellulases produced by *Trichoderma reesei*, the biotechnological workhorse of the genus, are important industrial products, especially with respect to production of second generation biofuels from cellulosic waste. Genetic engineering not only led to significant improvements in industrial processes but also to intriguing insights into the biology of these fungi and is now complemented by the availability of a sexual cycle in *T. reesei*/*Hypocrea jecorina*, which significantly facilitates both industrial and basic research. This review aims to give a broad overview on the qualities and versatility of the best studied *Trichoderma* species and to highlight intriguing findings as well as promising applications.

Keywords *Trichoderma* · *Hypocrea* · Cellulase · Biocontrol · Heterologous protein expression · Emerging human pathogen · Green mold disease · Biodiversity · Application · Biofuels

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Biodiversity and phylogeny of *Trichoderma*

The first description of a fungus named *Trichoderma* dates back to 1794 (Persoon 1794), and in 1865, a link to the sexual state of a *Hypocrea* species was suggested (Tulasne and Tulasne 1865). However, the different species assigned to the genus *Trichoderma*/*Hypocrea* were difficult to distinguish morphologically. It was even proposed to reduce taxonomy to only a single species, *Trichoderma viride*. Hence, it took until 1969 that development of a concept for identification was initiated (Rifai 1969; Samuels 2006). Thereafter, numerous new species of *Trichoderma*/*Hypocrea* were discovered, and by 2006, the genus already comprised more than 100 phylogenetically defined species (Druzhinina et al. 2006a). In some cases, especially in earlier reports, misidentifications of certain species occurred, for example with the name *Trichoderma harzianum* which has been used for many different species (Kullnig et al. 2001). However, it is difficult to safely correct these mistakes without analyzing the strains originally used, and therefore, we describe the data using the names as originally reported. In recent years, safe identification of new species was significantly facilitated by

development of an oligonucleotide barcode (TrichOKEY) and a customized similarity search tool (TrichoBLAST), both available online at www.isth.info (Druzhinina et al. 2005; Kopchinskiy et al. 2005). A further useful tool for characterization of newly isolated *Trichoderma* species (but also recombinant strains) are phenotype microarrays, which allow for investigation of carbon utilization patters for 96 carbon sources (Bochner et al. 2001; Kubicek et al. 2003; Druzhinina et al. 2006b). The continued efforts to elucidate diversity and geographical occurrence of *Trichoderma*/*Hypocrea* resulted in detailed documentations of the genus in Europe and worldwide (Samuels et al. 2002a; Chaverri and Samuels 2003; Jaklitsch 2009; http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm)

The Index Fungorum database (http://www.indexfungorum.org/Names/Names.asp) currently even lists 471 different names for *Hypocrea* species and 165 records for *Trichoderma*. However, many of these names have been introduced long before molecular methods for species identification were available and thus are likely to have become obsolete in the meantime. At present, the International Subcommission on *Trichoderma*/*Hypocrea* lists 104 species (http://www.isth.info/biodiversity/index.php), which have been characterized at the molecular level. Seventy-five species of *Hypocrea* have been identified in temperate Europe (Jaklitsch 2009). Nevertheless, a considerable number of putative *Hypocrea* strains and even more *Trichoderma* strains, for which sequences have been deposited in GenBank, are still without safe identification (Druzhinina et al. 2006a) and remain to be studied further. Species of the genus produce a broad array of pigments from bright greenish-yellow to reddish in color, although some are also colorless. Similarly, conidial pigmentation varies from colorless to various green shades and sometimes also gray or brown. Other than pigmentation, species identification within the genus is difficult because of the narrow range of variation of the simplified morphology in *Trichoderma* (Gams and Bissett 1998).

Characteristics of *Trichoderma* spp.

Trichoderma spp. are ubiquitous colonizers of cellulosic materials and can thus often be found wherever decaying plant material is available (Kubicek et al. 2008; Jaklitsch 2009) as well as in the rhizosphere of plants, where they can induce systemic resistance against pathogens (Harman 2000). Some characteristic features of different *Trichoderma* spp. are shown in Fig. 1. The search for potent biomass degrading enzymes and organisms also led to isolation of these fungi from unexpected sources, such as cockroaches (Yoder et al. 2008), marine mussels and shellfish (Sallenave et al. 1999; Sallenave-Namont et al. 2000), or termite guts (Sreerama and Veerabhadrappa 1993). *Trichoderma* spp. are characterized by rapid growth, mostly bright green conidia and a repetitively branched conidiophore structure (Gams and Bissett 1998).

Despite the early suggested link between *Trichoderma* and *Hypocrea* (Tulasne and Tulasne 1865), this anamorph–teleomorph relationship was only confirmed more than 100 years later for *Trichoderma reesei* and *Hypocrea jecorina* (Kuhls et al. 1996). Nevertheless, *T. reesei* was then termed a clonal, asexual derivative of *H. jecorina* because all attempts to cross the available strains of this species had failed. It took more than a decade until a sexual cycle was reported in any *Trichoderma* species (Seidl et al. 2009a), and a detailed study on molecular evolution of this species led to the discovery of a described sympatric agamospecies *Trichoderma parareesei* (Druzhinina et al. 2010). Especially because of the industrial importance of *T. reesei*, the availability of a sexual cycle was a groundbreaking discovery and now paves the way for elucidation of sexual development also in other members of the genus.

Trichoderma spp. are highly successful colonizers of their habitats, which is reflected both by their efficient utilization of the substrate at hand as well as their secretion capacity for antibiotic metabolites and enzymes. They are able to deal with such different environments as the rich and diversified habitat of a tropical rain forest as well as with the dark and sterile setting of a biotechnological fermentor or shake flask. Under all these conditions, they respond to their environment by regulation of growth, conidiation, enzyme production, and hence adjust their lifestyle to current conditions, which can be exploited for the benefit of mankind. One of these environmental factors is the presence or absence of light. *Trichoderma* has a long tradition of research toward the effect of light on its physiology and development, which already started in 1957 and largely paralleled that of *Phycomyces blakesleeanus* (Schmoll et al. 2010). Besides effects on growth, reproduction, and secondary metabolite biosynthesis, which are common light responses in fungi, also a surprising influence of light on cellulase gene expression has been found (Schmoll et al. 2005). This link between light response and metabolic processes was further substantiated by a study on carbon source utilization using phenotype microarrays in light and darkness (Friedl et al. 2008). Studies on the molecular basis of these light effects revealed that interconnections between the signaling pathways of light response, heterotrimeric G-proteins, the cAMP-pathway, sulfur metabolism, and oxidative stress are operative in *Trichoderma* (Schmoll et al. 2010; Tisch and Schmoll 2010).

In recent years, research with *Trichoderma* has been facilitated significantly by sequencing of the genomes of three strains representing the most important applications of this genus: The genome sequence of *T. reesei*, the industrial workhorse (Martinez et al. 2008; http://genome.jgi-psf.org/

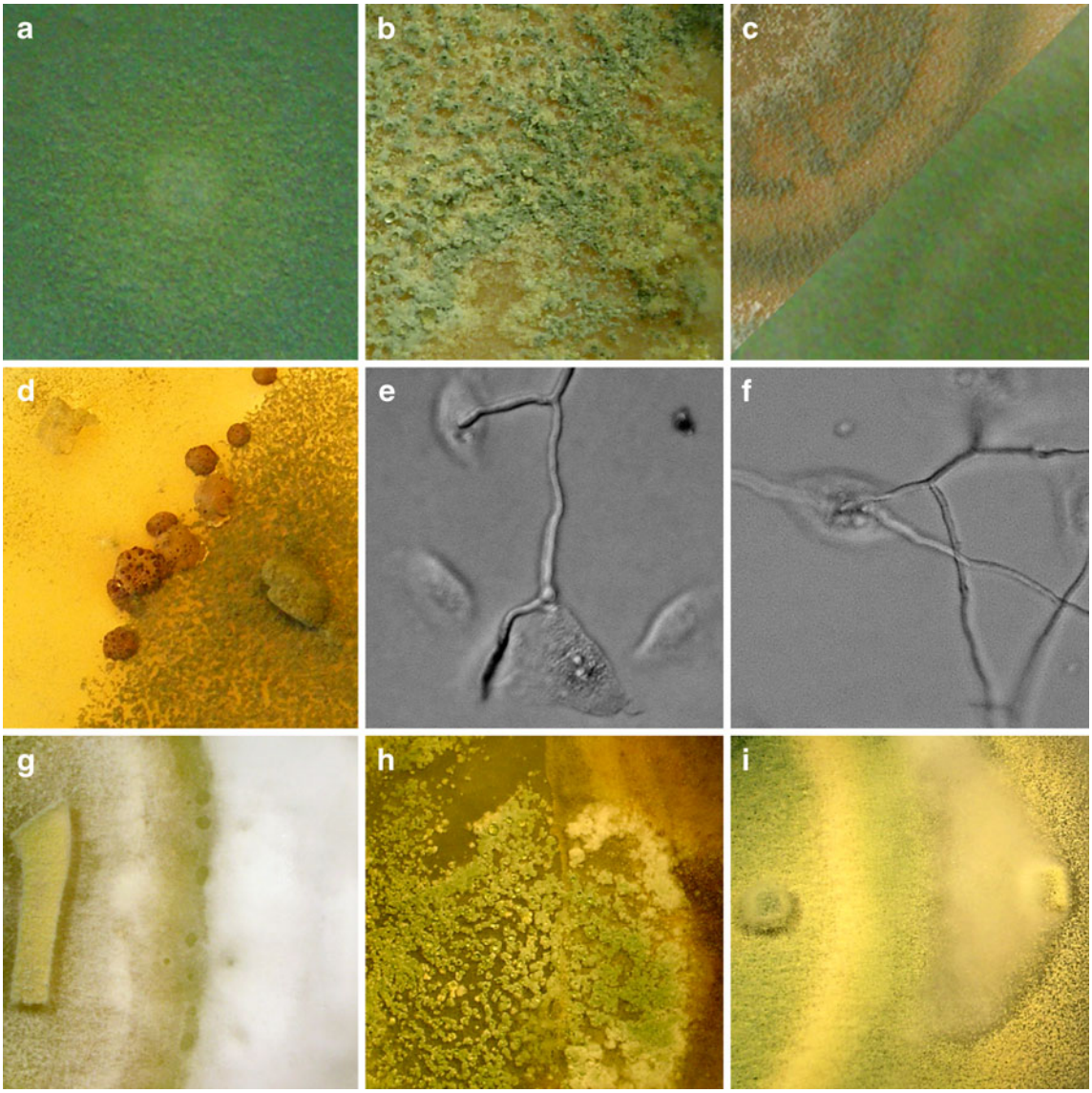

Fig. 1 Characteristic features of *Trichoderma* spp. **a** *T. reesei* and **b** *T. atroviride* growing on plates, **c** *T. reesei* or *H. jecorina* growing in daylight and showing light responsive conidiation, **d** fruiting body formation of *T. reesei* upon crossing with a nature isolate of *H. jecorina*, (**e**, **f**) *T. longibrachiatum* germinating and growing on human cells, **g**, **i** *T. reesei* (*left*) during confrontation with *Pythium ultimum* (*right*), **h** *T. atroviride* (*left*) during confrontation with *R. solani* (right)

Trire2/Trire2.home.html), surprisingly revealed that, despite its importance in industrial cellulase production, its genome comprises the fewest amount of genes encoding cellulolytic and hemicellulolytic enzymes. Analysis and annotation of the genomes of *Trichoderma atroviride* and *Trichoderma virens*, two important biocontrol species (http://genome.jgi-psf.org/Triat1/Triat1.home.html; http://genome.jgi-psf.org/Trive1/Trive1.home.html), is still in progress. Interestingly, the genomes of *T. atroviride* and *T. virens* are significantly larger than that of *T. reesei*, and they comprise roughly 2000 genes more than does *T. reesei*. It will be interesting to learn the significance of this considerable difference in genome sizes in the physiology of these fungi. These milestones in research with *Trichoderma* enabled detailed studies, which provided intriguing insights into their lifestyle, physiology, and the underlying mechanisms at the molecular level (Brunner et al. 2008; Martinez et al. 2008; Schmoll 2008; Le Crom et al. 2009; Seidl et al. 2009b).

Tools for genetic manipulation of *Trichoderma*

Due to the industrial application of *T. reesei*, the genetic toolkit for this fungus is the most extensive of the genus, although also research with other species is not limited by technical obstacles and most tools can also be used for all

species with slight modifications. Transformation of many species is possible, and different approaches such as protoplasting (Gruber et al. 1990), *Agrobacterium*-mediated transformation (Zeilinger 2004), or biolistic transformation (Lorito et al. 1993) were developed. The range of selectable marker cassettes, which includes hygromycin (Mach et al. 1994) and benomyl resistance (Peterbauer et al. 1992; Schuster et al. 2007), the *Aspergillus nidulans amdS* gene, which enables growth on acetamide as sole nitrogen source (Penttila et al. 1987) as well as the auxotrophic markers, *pyr4* (Gruber et al. 1990), *arg2* (Baek and Kenerley 1998), and *hxk1* (Guangtao et al. 2010) allows for construction of multiple mutants, which is now facilitated by the availability of a *T. reesei* strain with perturbed nonhomologous end-joining pathway (Guangtao et al. 2009). Sequential deletions despite a limited number of selection markers became possible by the use of a blaster cassette comprising direct repeats for homologous recombination and excision of the marker gene (Hartl and Seiboth 2005). Besides knockout strategies for functional analysis of genes, also expression of antisense constructs for knockdown (Rocha-Ramirez et al. 2002; Moreno-Mateos et al. 2007; Schmoll et al. 2009) was reported for *Trichoderma*, and RNAi has been shown to function in *T. reesei* (Brody and Suchindra 2009). Last but not least, the recent discovery of a sexual cycle in *T. reesei* (Seidl et al. 2009a) further boosts the versatility of this fungus for research and industry.

Defense mechanisms and their exploitation

Successful colonization of a given habitat by any organism is crucially dependent on its potential to defend its ecological niche and to thrive and prosper despite competition for nutrients, space, and light. Many fungi and especially those of the genus *Trichoderma* are masters of this game (Herrera-Estrella and Chet 2004; Harman 2006; Vinale et al. 2008). Their defense mechanisms comprise both enzymatic and chemical weapons, which make *Trichoderma* spp. efficient mycoparasites, antagonists, and biocontrol agents—characteristics that can be exploited by using *Trichoderma* spp. or the metabolites secreted by these fungi as biological fungicides to fight plant diseases caused by pathogenic fungi (Spiegel and Chet 1998; Vinale et al. 2006; Navazio et al. 2007; Vinale et al. 2009). Thereby *Trichoderma* spp. play an important role in the three-way interaction with the plant and the pathogen (Lu et al. 2004; Woo et al. 2006).

Trichoderma's strategies for combat

After publication of *Trichoderma lignorum* (later found to be *T. atroviride*) acting as a parasite on other fungi in 1932 (Weindling 1932), research on antagonistic properties of *Trichoderma* spp. progressed rapidly. Nowadays, the most important species in this field are *T. atroviride* (in earlier reports sometimes misidentified as *T. harzianum*), *T. harzianum*, *T. virens*, and *Trichoderma asperellum* (Benitez et al. 2004), while *T. reesei*, the biotechnological workhorse, can rather be seen as a model organism used because of the established molecular biological methods and available recombinant strains (Seidl et al. 2006). *Trichoderma* spp. are able to control ascomycetes, basidiomycetes, and oomycetes (Monte 2001; Benitez et al. 2004), and recently, also their effect on nematodes was reported (Dababat et al. 2006; Kyalo et al. 2007; Goswami et al. 2008)

In their defensive actions, *Trichoderma* spp. apply lytic enzymes (Kubicek et al. 2001; Viterbo et al. 2002), proteolytic enzymes (Kredics et al. 2005; Suarez et al. 2007; Chen et al. 2009), ABC transporter membrane pumps (Ruocco et al. 2009), diffusible or volatile metabolites (Calistru et al. 1997; Eziashi et al. 2006), and other secondary metabolites (Reino et al. 2008) as active measures against their hosts or they succeed by their impairing growth conditions of pathogens (Benitez et al. 2004). Interestingly, the success of these actions is not independent of the surrounding temperature (Mukherjee and Raghu 1997), which can be crucial for the use as a biocontrol agent in different climates. Large-scale studies on gene expression during biocontrol at least in part reflect these findings (Grinyer et al. 2005; Marra et al. 2006; Samolski et al. 2009) and reveal additional components with potential effectivity such as a superoxide dismutase (Grinyer et al. 2005) and amino acid oxidase (Tseng et al. 2008) to be secreted under these conditions. Moreover, the response of *Trichoderma* to its host has also been shown to involve stress response, response to nitrogen shortage, cross pathway control, lipid metabolism, and signaling processes (Seidl et al. 2009b).

Regulatory mechanisms triggering the defense of *Trichoderma*

Signal transduction pathways triggering the genes involved in biocontrol and mycoparasitism have been studied in considerable depth and include heterotrimeric G-protein signaling, mitogen-activated protein kinase (MAPK) cascades, and the cAMP pathway (Zeilinger and Omann 2007). Especially the MAP-kinase TVK1, characterized in *T. virens* (Mendoza-Mendoza et al. 2003; Mukherjee et al. 2003; Mendoza-Mendoza et al. 2007) as well as its orthologs in *T. asperellum* (TmkA; Viterbo et al. 2005) and *T. atroviride* (TMK1; Reithner et al. 2007), is important in regulation of signaling mechanisms targeting output pathways relevant for efficient biocontrol. Transcript levels of the respective genes increased upon interaction with

plant roots in *T. virens* and *T. asperellum* (Viterbo et al. 2005). Deletion of *T. atroviride tmk1* causes higher antifungal activity and improved protection against *Rhizoctonia solani* but reduced mycoparasitic activity (Reithner et al. 2007). In agreement with this study, lack of *T. virens* TVK1 considerably increases biocontrol effectivity of this fungus (Mendoza-Mendoza et al. 2003). Hence, although deletion of the respective genes causes reduced mycoparasitic efficiency, the biocontrol abilities of the mutant strains are enhanced.

As for the action of the pathway of heterotrimeric G-protein signaling, two genes have been studied so far with respect to biocontrol related mechanisms in *Trichoderma* spp.: the class I (adenylate cyclase inhibiting) G-alpha subunits TGA1 of *T. atroviride* and TgaA of *T. virens* as well as the class III (adenylate cyclase activating) G-alpha subunits TGA3 of *T. atroviride* and GNA3 of *T. reesei*. TGA1 plays an important role in regulation of coiling around host hyphae and regulates production of antifungal metabolites. Lack of TGA1 results in enhanced growth inhibition of host fungi (Rocha-Ramirez et al. 2002; Reithner et al. 2005). For TgaA, a host specific involvement as shown in case of the action of MAP-kinases has been reported (Mukherjee et al. 2004). TGA3 on the other hand is crucial for biocontrol since deletion of the corresponding gene resulted in avirulent strains (Zeilinger et al. 2005). Since constitutive activation of GNA3 in *T. reesei* is suggested to positively influence mycoparasitism, a similar mechanism, may be at work in this fungus (Silva et al. 2009). These results are in agreement with analysis of cAMP signaling components, which indicate a positive role of cAMP in biocontrol (Mukherjee et al. 2007). Recently, also an important role in biocontrol of *T. virens* has been reported for the homolog of the VELVET protein, so far mainly known as light-dependent regulator protein (Mukherjee and Kenerley 2010).

Attempts were made to identify characteristics among all these genes and enzymes regulated upon interaction of *Trichoderma* with a pathogen, which could be used to distinguish efficient from nonefficient biocontrol strains isolated from nature (Nagy et al. 2007; Scherm et al. 2009). However, only further, extensive studies will reveal the reliability of standardized marker gene assays for evaluation of potential biocontrol strains.

Trichoderma as a protector of plant health

The beneficial action of *Trichoderma* spp. is not limited to fighting pathogens; they have also been shown to be opportunistic plant symbionts, enhancing systemic resistance of plants (Yedidia et al. 1999; Shoresh et al. 2010), a response which is improved by ceratoplatanin family proteins (Djonovic et al. 2006; Seidl et al. 2009b). Perception of the signals transmitted by *Trichoderma* in the plant requires the function of a MAPK (Shoresh et al. 2006), and also in the fungus itself, a MAPK signaling is crucial for full induction of systemic response in the plant (Viterbo et al. 2005). By colonizing plant roots, which is significantly enhanced by swollenin (Brotman et al. 2008) or invading them, they are also carried through soil and occupy new niches. This interaction with plants as well as their rhizosphere competence leads to enhanced root proliferation, better growth, and protection of the plants against toxic chemicals, against which *Trichoderma* spp. themselves show a remarkable resistance. Hence, these fungi are promising agents that can be applied for remediation of polluted soil and water by treatment of appropriate plants with spores (Harman et al. 2004).

Secondary metabolites

In order to survive and compete in their ecological niche, fungi apply not only enzymatic weapons but also have a potent arsenal for chemical warfare at their disposal (Vinale et al. 2008). Thereby, not only potential antibiotics (for example the peptaibols) but also mycotoxins and more than 100 metabolites with antibiotic activity including polyketides, pyrones, terpenes, metabolites derived from amino acids, and polypeptides (Sivasithamparam and Ghisalberti 1998) were detected in *Trichoderma* spp. and have been suggested to be used for chemotaxonomy of these species. However, the evolution of peptaibol formation seems to be too complex to allow for prediction of peptaibol production profiles form phylogenetic relationships (Daniel and Filho 2007; Kubicek et al. 2007; Neuhof et al. 2007; Degenkolb et al. 2008). One of the first characterized secondary metabolites of *Trichoderma* spp. was the peptide antibiotic paracelsin (Bruckner and Graf 1983; Bruckner et al. 1984). A wide variety of peptaibols was identified in *Trichoderma* thereafter (Degenkolb et al. 2003, 2007, 2008; Stoppacher et al. 2008). Interestingly, the four trichothecene mycotoxin-producing species (*Trichoderma brevicompactum*, *Trichoderma arundinaceum*, *Trichoderma turrialbense*, and *Trichoderma protrudens*) are not closely related to those species used in biocontrol, which not only means that the application of biocontrol in agriculture does not pose a risk in this respect but also indicates that these mycotoxins do not play a major role in the defense mechanisms of these fungi (Nielsen et al. 2005; Degenkolb et al. 2008). As many other fungi, also *Trichoderma* spp. have been shown to produce a broad array of volatile organic compounds, which recently have received closer attention (Stoppacher et al. 2010). With respect to regulation of peptaibol biosynthesis in *Trichoderma* spp., several

factors are known to be relevant. Environmental cues such as light, pH, nutrients, starvation, or mechanical injury impact this process. Efficient production of peptaibols predominantly occurs in solid cultivation and correlates with conidiation (Kubicek et al. 2007; Tisch and Schmoll 2010). Signaling molecules involved range from the blue light photoreceptors BLR1 and BLR2 to the G-alpha subunits GNA3 (TGA3) and GNA1 as well as protein kinase A (Reithner et al. 2005; Komon-Zelazowska et al. 2007a). Thereby, GNA3 is essential for peptaibol formation, but on the other hand, stimulation of peptaibol formation in the absence of BLR1 and BLR2 is still possible (Komon-Zelazowska et al. 2007a).

Trichoderma spp. as industrial workhorses

Shortly after the discovery of *T. viride* QM6a by the US army during World War II (Reese 1976), the outstanding efficiency of its cellulases led to extensive research toward industrial applications of these enzymes. Later on, this species was renamed *T. reesei* in honor of Elwyn T. Reese (Simmons 1977) and became the most important cellulase producer worldwide. Until now, this species is the most important one of the genus for industrial purposes.

Cellulases and plant cell wall-degrading enzymes

Rising energy costs and the imminent climate change led to an increased attention to biofuel production (Somerville 2007; Rubin 2008). As a potent cellulase producer, research with *T. reesei* is nowadays particularly focused on improvement of efficiency of the enzyme cocktail produced in order to decrease overall costs of production of bioethanol from cellulosic waste material (Kumar et al. 2008), although applications in the pulp and paper industry (Buchert et al. 1998) and textile industry (Galante et al. 1998a) are also important. After the early mutation programs (El-Gogary et al. 1998) and strain improvement, the protein secretion capacity of industrial strains now reaches 100 g/l, with up to 60% of the major cellulase Cel7a (CBHI) and 20% of Cel6a (CBHII). High levels of cellulase and hemicellulase gene expression can be achieved upon cultivation on cellulose, xylan, or a mixture of plant polymers (Mach and Zeilinger 2003) as well as on lactose (Seiboth et al. 2007), all of which are agricultural or industrial byproducts. The natural inducer of at least a subset of these enzymes is believed (yet not definitely proven) to be sophorose, a transglycosylation product of cellobiose (Sternberg and Mandels 1979; Vaheri et al. 1979). Targeted strategies to further enhance the efficiency of the enzymes secreted include elucidation of regulatory mechanisms both at the promotor level (Mach and Zeilinger 2003; Schmoll and Kubicek 2003) as well as with respect to signal transduction (Schmoll et al. 2010). However, auxiliary components acting on the substrate could also enhance efficiency of its degradation (Saloheimo et al. 2002; Schmoll and Kubicek 2005).

Metabolic engineering in recent years provided intriguing insights into these processes (Kubicek et al. 2009), and exploration of the genome sequence of *T. reesei* revealed that this industrial workhorse possesses the smallest amount of genes within Sordariomycetes encoding the enzymes which made it so popular—plant cell wall-degrading enzymes (Foreman et al. 2003; Martinez et al. 2008). Availability of the genome sequence also spurred genome wide analysis of early mutant strains and identification of putatively beneficial mutations, which caused their high efficiency (Le Crom et al. 2009). Interestingly, it seems that even early mutants such as RutC-30 bear considerable alterations of their genome (Seidl et al. 2008). These novel tools also facilitated characterization of the enzyme cocktails secreted by these strains (Herpoel-Gimbert et al. 2008). In addition to these efforts enzyme engineering approaches (Bansal et al. 2009), improvement of the secretion machinery (Conesa et al. 2001; Kruszewska et al. 2008) as well as screening of the enormous variety of plant cell wall-degrading enzymes from nature isolates (Kubicek et al. 1996) or other organisms secreting cellulases (Dashtban et al. 2009) and directed evolution (Nakazawa et al. 2009) complement the optimization of the regulatory mechanism of available production strains. Hence, with the aid of *Trichoderma*, economically reasonable production of second generation biofuels from waste products is on the way.

Heterologous protein production

Filamentous fungi are versatile cell factories and frequently used for heterologous protein expression (Adrio and Demain 2003), especially if they have generally regarded as safe status (Nevalainen et al. 2005), as has *T. reesei* (Nevalainen et al. 1994). The industrial use of *T. reesei* as a producer of heterologous proteins started more than 20 years ago with the production of calf chymosin (Harkki et al. 1989; Uusitalo et al. 1991). Shortly thereafter, even expression of immunologically active antibody fragments (Nyyssonen et al. 1993) in *T. reesei* was achieved and numerous enzymes and performance proteins followed. Nowadays, *T. reesei* is one of the most commonly used filamentous fungi for heterologous protein production (Penttila 1998; Nevalainen et al. 2005).

Based on the efficient expression as well as the considerable knowledge on regulation of cellulase genes, their promotors are routinely used for heterologous protein production (Penttila 1998; Schmoll and Kubicek 2003). Consequently, improvements in cellulase transcription are

also beneficial for these applications. In many cases, the signal peptide of Cel7a (CBHI) is used to facilitate efficient secretion of the product into the culture medium. Nevertheless, also alternative promotors were also shown to be useful for certain applications (Keränen and Penttilä 1995). In general, the high efficiency and the inducibility of the cellulase promotors have proven beneficial in many applications. Using the cellulase promotors, also relatively cheap carbon sources such as cellulose or lactose can be used for production. Nevertheless, it must be considered that the large amount of enzymes secreted into the culture medium can be an issue in specific purification of the heterologous protein, and the complex substrates used could induce extracellular proteases, which are deleterious for the yield of the process (Keränen and Penttilä 1995). For further improvement, promotor modifications, for example with the *chb1*-promotor (Liu et al. 2008), can increase yields of the protein to be expressed.

Food industry

With their long history of safe industrial scale enzyme production, *Trichoderma* spp. have also been extensively applied for production of food additives and related products (Nevalainen et al. 1994; Blumenthal 2004). Currently, various *Trichoderma* enzymes are applied to improve the brewing process (β-glucanases), as macerating enzymes in fruit juice production (pectinases, cellulases, hemicellulases), as feed additive in livestock farming (xylanases) and for pet food. Cellulases are mainly applied in baking, malting, and grain alcohol production (Galante et al. 1998b). However, not only enzymes but also metabolites of *Trichoderma* spp. are used as additives. One of the first products isolated from *T. viride* was a chemical with characteristic coconut-like aroma, a 6-pentyl-α-pyrone with antibiotic properties, the production of which was constantly improved to reach concentrations of more than 7 g/L in extractive fermentation cultures in *T. atroviride* nowadays (Collins and Halim 1972; Oda et al. 2009). An interesting idea is the application of cell wall-degrading enzymes, for example of *T. harzianum*, as food preservatives because of their antifungal effect (Fuglsang et al. 1995), but so far this suggestion has not found broad application. With a similar aim, *T. harzianum* mutanase can be used in toothpaste to prevent accumulation of mutan in dental plaque (Wiater et al. 2005).

Black sheep in the genus *Trichoderma*

In addition to the highly beneficial and frequently used species, the genus *Trichoderma* also comprises opportunistic human pathogens, which show efficient growth at body temperature and mycoparasitic species, which are a significant threat to mushroom farms.

Human pathogenic species

Besides such long-known and well-studied pathogenic fungi as *Candida*, *Aspergillus*, or *Crypotcoccus*, also the genus *Trichoderma* comprises opportunistic human pathogens, which pose a serious and often lethal threat—especially to HIV-infected persons and other immunocompromised patients. Belonging to the emerging fungal pathogens, these fungi are often not recognized or diagnosed in a stadium when efficient treatment is problematic (Walsh et al. 2004). *Trichoderma* species have been reported to cause respiratory problems due to volatile organic compounds they produce (Larsen et al. 1998), but more importantly, they can infect immunocompromised patients (*Trichoderma citrinoviride*, *T. harzianum*, and *Trichoderma longibrachiatum* and *Hypocrea orientalis*) after transplantations or suffering from leukemia or HIV (Kredics et al. 2003). The typically poor prognosis of such infections is (besides delayed diagnosis) predominantly due to the low susceptibility of these fungi to commonly used antifungal agents (Chouaki et al. 2002; Kratzer et al. 2006), which often necessitates combined treatment with different drugs (Kratzer et al. 2006; Alanio et al. 2008). Nevertheless, few data on investigation of virulence factors of these fungi are available (Kredics et al. 2004). Among the clinical isolates, *T. longibrachiatum* and *H. orientalis* are the most common ones. Interestingly, no specific phylogenetic characteristics of the clinical isolates as compared to environmental isolates could be found, and no correlation between virulence or pathogenicity and genomic structure was detected (Antal et al. 2005, 2006; Druzhinina et al. 2008). However, most intriguingly, *T. longibrachiatum* not only causes disease; at the same time, it seems to be a source for potential antifungal drugs efficient against *Candida* and *Aspergillus* species (Vicente et al. 2001).

Green mold disease

Cultivation of the edible mushrooms *Agaricus bisporus* (champignon) and *Pleurotus ostreatus* (oyster mushrooms) on mushroom farms all over the world is of considerable economic importance. In the 1980s, a mixture of strains at first identified as *T. harzianum* was found to cause deleterious infections in these farms (Seaby 1987) with losses between 30% and 100% (Seaby 1998). Actually, these strains represented two new species, *Trichoderma aggressivum* fsp. *europeae* and *T. aggressivum* fsp. *aggressivum* (Samuels et al. 2002b). Since then, this "green mold disease" has spread all over the world (Komon-Zelazowska

et al. 2007b) and was shown to be mainly caused by *Trichoderma pleurotum*, *Trichoderma pleuroticola* in *P. ostreatus* (Park et al. 2006), and *T. aggressivum* in *A. bisporus*. Nevertheless, also *T. harzianum*, *T. longibrachiatum*, *Trichoderma ghanense*, *T. asperellum*, and *T. atroviride* have been detected in *Agaricus* compost and *Pleurotus* substrates (Hatvani et al. 2007), but aggressive colonization of the substrate has not been proven for these species. This threat to commercial mushroom production has recently also led to the development of methods for rapid and specific detection of these fungi in cultivation substrates (Kredics et al. 2009). A similar objective led to the development of a key for identification of *Trichoderma* species commonly associated with commercially grown mushrooms (Muthumeenakshi et al. 1998; Samuels et al. 2002b).

Future prospects

More than ever before sustainable economy and protection of our environment are dominant topics in our everyday life and one alarming report about contaminated landscapes or catastrophes caused by climate change follows another. Today, 87% of energy used in the world comes from nonrenewable sources like natural gas, oil, and coal (Merino and Cherry 2007). Although biofuel production is now being pushed in order to decrease the requirement for fossil fuels, the raw materials therefore originate from commodities and land also needed for food. In this respect, production of the so-called second generation biofuels from agricultural waste products by the aid of cellulases and hemicellulases produced for example by *T. reesei* and fermentation of the resulting oligosaccharides by yeast provides an alternative strategy. However, for an economically competitive process an increase in efficiency of more than 40-fold would be necessary, which is a formidable challenge for research with *Trichoderma*.

Sustainability is also the major driving force for investigation of biocontrol with *Trichoderma*. As opportunistic plant symbionts and effective mycoparasites, numerous species of this genus have the potential to become commercial biofungicides. The challenge in this field of research will be the development of reliable screening techniques, which allow for prediction of the biocontrol efficiency of a given isolate by determination of the key factors for this process. Nevertheless, also the ecological effects of widespread application of a single (or few) fungal species in agriculture remain to be investigated in order to ensure a truly beneficial effect for the environment.

Besides these major applications of *Trichoderma* spp., also the fields of green and white biotechnology become increasingly important for environmentally safe production of enzymes and antibiotics. These industrial applications will also benefit from studies on molecular physiology and regulatory processes, which continuously reveal novel and valuable metabolites and enzymes as well as components to be modified or adjusted for cost effective high yield production.

Last but not least, the extensive studies on diverse physiological traits available and still progressing for *Trichoderma* make these fungi versatile model organisms for research on both industrial fermentations as well as natural phenomena.

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