12 Ecological Genomics of Mycotrophic Fungi

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I. Introduction

A key feature of fungal communities—their interdependence with other organisms—is explained by their inability of primary production (heterotrophy). In consequence, fungi cannot form separate self-sustaining communities, and their occurrence is irrevocably linked with that of organisms on which they depend for their nutrition (Hawksworth and Mueller 2005). Contemporary interactions of fungi with plants derived from initially saprotrophic living of early fungi on dead algal material in periodic dry, limnetic ecosystems. It is conceivable that some of these fungi may have formed mutualistic associations with early terrestrial algae, which later gave rise to complex symbioses between high fungi and vascular plant modern algae. A phylogenomic study of pectinase gene expansions demonstrated that the early group of true fungi Chytridiomycota diverged from its sister clade and thus leading to the high fungi Dikarya only after pectin evolved in plant cell walls that happened not earlier than 750 million years ago (Mya) (Chang et al. 2015).

The establishment of interactions between fungi and other opisthokonts (nucleariids, other fungi and animals) is definitely more ancient than their relationships with plants and dates back to the origin of fungi as an entire monophyletic group. The divergence of the plant–animal–fungal lineages occurred likely 820–1200 Mya. Recent recalibrations of the most important fungal fossils and the construction of molecular clock phylogenetic trees allowed to put fungal evolution on a right

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track with the origin and diversification of other major lineages of multicellular eukaryotes (Lücking et al. 2009).

II. Obligate Intracellular Mycoparasitism in Cryptomycota

The availability of genomic and phylogenomic techniques shed light on the interactions between lineages of early fungi. Such studies included microsporidia (single-celled spore-forming endopathogens of animals, *Microspora*) with the ancient and relatively newly recognized group of Cryptomycota including *Rozella allomycis* (Fig. 12.1), the endoparasite of water mold (James et al. 2013; Jones et al. 2011). The latter fungus serves as the most important source of information as it is the only clade member that grows in culture. *R. allomycis* is an obligate endoparasite of the water mold fungus *Allomyces* (Blastocladiomycota) that grows as a naked mitochondriate protoplast capable of phagocytosis to devour the cytoplasm of its host. (Held 1980; James and Berbee 2012; Powell 1984) showed that *R. allomycis* has a fungal-specific chitin synthase and its resting sporangia contain chitin in cell walls. They thus conclude that Cryptomycota and *Rozella* are not evolutionary intermediates as it was previously assumed but are rather the divergent fungi that evolved from an ancestor that already had a complete suite of classical fungal characteristics.

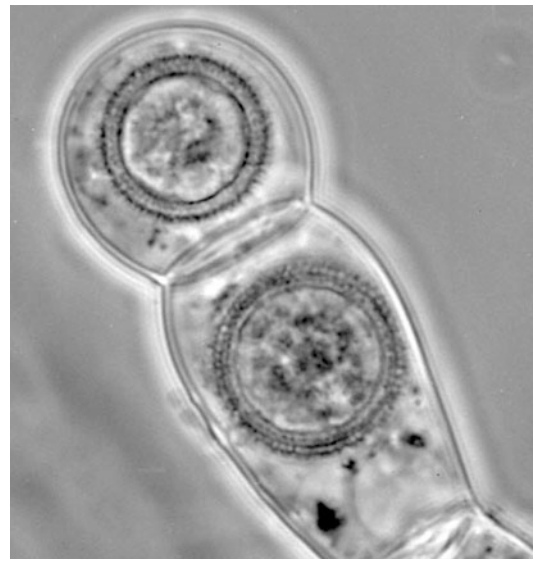

Fig. 12.1 *Rozella allomycis* parasitizing the chytrid *Allomyces*. Permission obtained from (Bruns 2006)

Genome sequencing of *R. allomycis* revealed insights into the previously unfeasible nature of its interactions with its host (James et al. 2013): it is diploid and contains 6350 predicted gene models. It includes four chitin synthases, one of which (division II chitin synthase) is specific for fungi and microsporidia (Ruiz-Herrera and Ortiz-Castellanos 2010). Interestingly, among the division II chitin synthases of *R. allomycis*, one contains a myosin domain, a feature that may be required for the polarized growth during invasion of *Allomyces*, a mechanism similar to the development of the penetration tube in corn smut Ustilaginales (Basidiomycota) (Schuster et al. 2012). James et al. (2013) used Oregon Green 488 conjugate of wheat germ agglutinin fluorescent stain that binds to *N*-acetylglucosamine residues and demonstrated that the infective cyst of *R. allomycis* contains this chitin precursor and that the chitin stain is most intense at those points where it penetrates the hyphae of *Allomyces*. A comparison of Cryptomycota including microsporidia with aphelids (Aphelidea, Opisthokonta) provided further insights into the interaction between the parasites and their hosts. Microsporidia and aphelids were previously considered as endoparasitic protozoans, and their placement within fungi only recently proposed (James et al. 2006; Karpov et al. 2013) and finally confirmed by phylogenomic analysis of a concatenated matrix of 200 gene sequences (James et al. 2013). Moreover, they found that *R. allomycis* genome contains orthologs of the three genes that were previously considered to be only present in microsporidian genomes and were thus interpreted as incidences of horizontal gene transfer (HGT) to serve the needs of intracellular parasitism (Cuomo et al. 2012). These are genes encoding a nucleotide phosphate transporter (NTTs; Pfam PF03219), a nucleoside H^+ symporters (PANDIT PF03825), and a chitinase class I genes (Pfam PF00182). The identification of these genes in *Rozella* represents an independent line of evidence for a close evolutionary link between Cryptomycota and microsporidia and indicates shared signatures of energy parasitism in the form of nucleotide and nucleoside transporters and genes for chitin degradation. Importantly, NTP transporters are involved in a specific theft of ATP from the host in microsporidia and the intracellular parasitic prokaryotes *Chlamydia* (*chlamydiae*) from which the genes were originally obtained by HGT (James et al. 2013; Tsaousis et al. 2008). Interestingly, the mitochondrial genome of *Rozella* showed features of degeneration that supports the hypothesis that the capacity to import ATP results in drastic genome changes for the mitochondrion. Sim-

ilar findings were also made in microsporidia, in which the capacity to retrieve ATP from their hosts by the HGT-derived bacterial NTTs is linked with a severe degeneration of their mitochondrion to a vestigial, genome-less organelle called a mitosome (Williams et al. 2002). Analysis of *Rozella*'s proteome and secretome, respectively, mainly revealed adaptations to endoparasitism that are convergent to those in other lineages of single-celled eukaryotes with a similar lifestyle. As expected for an obligate intracellular pathogen, the *Rozella* proteome is missing key components of primary metabolism. Overall, the portion of the proteome responsible for primary metabolism of *Rozella* is more similar to that of the apicomplexan parasites, *Plasmodium* and *Toxoplasma*, than that of microsporidia or other fungi. On the other hand, the amino acid metabolism of *R. allomycis* is more similar to that of Metazoa and Amoebozoa, perhaps suggestive of a phagotrophic mode of protein consumption and amino acid extraction. However, proteins involved in protein–protein interactions (e.g., signal transduction, protein folding, protein kinases, and proteins with WD40 domains) are all enriched in the *R. allomycis* proteome. James et al. (2013) hypothesized that some of the protein–protein interaction domains are actually involved in the direct manipulation of host signaling or recycling of host proteins. In support of this argument, they identified 22 genes of the Crinkler family of effector proteins. Crinkler proteins are found in many symbiotic, microbial eukaryotes but are best known in oomycete plant pathogens as secreted proteins that translocate into the host cytoplasm or nucleus to induce plant cell death. Thus, these new and most advanced genomic studies clearly demonstrate the ancient nature of intimate interactions between fungal lineages. The existence of mainly obligate and endocellular parasites in Cryptomycota sensu lato and the obvious lack of less specialized facultative associations between organisms from early fungal lineages is probably best explained by the long evolutionary history in aquatic ecosystem. Consequently, such interactions are rare among high fungi, which, however, interact in a great diversity of ways.

III. Diversity of Interactions Between High Fungi

Even before the inclusion of Cryptomycota in true fungi, this kingdom was considered as one of the most diverse members of the eukaryotic domain being probably only second after Arthropoda (Animalia). Consequently the ecology of these organisms in general and the structures of fungal communities in particular are very complex. Unfortunately fungal ecosystems and interactions are frequently described by using the better established botanical (and rarely zoological) terminology that creates considerable confusion. The review of several inherent problems and ambiguities associated with terminologies used in general ecology to describe fungal interactions is made by Tuininga (2005). Based on the way how fungi receive nutrients, she proposed to divide interfungal interactions in nutritive and nonnutritive. So-called nutritive fungal interactions can then be further be differentiated into biotrophy (deriving nutrients from the cytoplasm of a living host) and necrotrophy or predation, i.e., rapid utilization of nutrients from an organism after killing it (Jeffries and Young 1994; Dighton et al. 2005; Atanasova et al. 2013). While necrotrophy is ultimately beneficial for one partner only (the host or the predator), biotrophic interactions may vary in their importance for the two fungi from mutualism (hypothetically assumed but almost not documented) to commensalism and classical parasitism.

A. Fungi that "Stick Together"

To the best of our knowledge, cases of interfungal mutualism are not well documented. Commensalism between fungi has been demonstrated in vitro although explanations for such observations are still insufficient. Deacon (2005), working with thermophilic fungi *Chaetomium* (Sordariales, Ascomycota) and *Thermomyces* (Eurotiales, Ascomycota), showed that the latter non-cellulolytic fungus clearly benefited from the ability of *Chaetomium* to degrade cellulose in the compost. When inoculated together, the two fungi could degrade more of the cellulose filter paper sample compared to *Chaetomium* alone. The advantages of *Chaetomium* from the presence of *Thermomyces* remain to be explained. Beneficial interactions between fungi were also shown by Friedl and Druzhinina (2012), studying infrageneric communities of *Trichoderma* (Hypocreales, Ascomycota) in vertical profiles of the two undisturbed soils in the Danube valley. They detected up to a dozen of *Trichoderma* species to coexist in a soil sample of not more than 200 mg.

Pairwise in vitro modeling of *Trichoderma* communities by cultivating one species on the culture filtrate of the other species and measuring the resulting fitness (growth rate and conidiation efficiency) revealed that many of such interactions provided a benefit, but cases of no effect or even inhibition of growth and/or conidiation were observed too. Our studies of *Trichoderma* molecular evolution and diversity in different habitats demonstrate frequent cases of sympatric speciation and cohabitation of sibling species that remains to be explained (Atanasova et al. 2010; Friedl and Druzhinina 2012; Hoyos-Carvajal et al. 2009; López-Quintero et al. 2013; Migheli et al. 2009). Besides these few examples, the absolute majority of described nutritional interactions between fungi are neither mutualistic nor based on commensalism. The aggressive behavior of fungi against each other is widely used in agriculture to suppress plant pathogenic fungi, but it may also cause adverse effects on mushroom farms and on fungal bioeffectors used for plant growth promotion such as arbuscular mycorrhizal fungi. We therefore first describe the types of such hostile interactions between fungi and then focus on several best studied cases.

B. Types of Hostile Interactions Between Fungi

"The term mycoparasitism applies strictly to those relationships in which one living *fungus* [*underlined by the authors*] acts as a nutrient source for another." This definition by Peter Jeffries (Jeffries 1995) that limits the term to the fungal kingdom is only one of numerous similar clear statements commonly present in books and articles (Barnett 1963; Deacon 2005; Gupta et al. 2014). Ideally, the strictness of the definition should limit the use of a term to appropriate cases. Unfortunately it is not always the case in fungal ecology as numerous interactions between fungi and fungi-like protozoans (e.g., Oomycota) are also referred as mycoparasitic (Ait Barka and Clément 2008; Benhamou et al. 1999; Gaderer et al. 2015; Rey et al. 2005; Vallance et al. 2009) due to the similar impact made by these and the true mycoparasitic interactions to plant pathology. Below we describe terms related to non-mutualistic interactions between fungi:

- **Fungivory, mycophagy, or mycotrophy**—the use of fungi for food. All three terms are synonymous and may be applied (1) to grazing on fungal hyphae (e.g., by mites or ants) or fruiting bodies (e.g., by deer or humans), (2) to various biotrophic interactions with fungi ranging from mutualism through commensalism and parasitism to predation, and (3) to saprotrophic nutrition on all types of dead fungal biomass. Fungivory nutrition is known for fungi, bacteria, plants, vertebrates (particularly for birds and mammals), invertebrates (gastropods, nematodes, and insects), and protozoans including fungi-like oomycetes and amoeba. Thus, when *Pythium* (Pythiales, Oomycota) attacks a fungus, this interaction may be referred as **mycophagy** (or fungivory or mycotrophy); in contrast, when a fungus attacks *Pythium*, another term should be used (e.g., parasitism).
- **Mycoparasitism**—the case of **mycophagy** when one fungus feeds on another fungus. It includes the true cases of parasitism when parasite does not kill its host. Such interactions are biotrophic are beneficial for a parasite (or a pathogen) and are harmful for the host.
  Necrotrophic mycoparasitism—the case of **mycophagy** that is best described as predation when the feeding fungus aims to kill its prey and then feed on its dead biomass. Some authors prefer to use "prey" and "predator," respectively, for simplicity and clarity (Atanasova et al. 2013; Barnett and Binder 1973; Druzhinina et al. 2011; Seidl et al. 2009). Necrotrophic mycoparasites tend to be more aggressive and unspecialized (Chet and Viterbo 2007). Biotrophic mycoparasites, on the other hand, are usually restricted to a certain host range and may also develop specialized structures to adsorb nutrients from their hosts. Some fungi may behave as biotrophic mycoparasites of some hosts, while in interactions with others they behave rather as predators (Zhang et al. 2015).
- **Hyperparasitism**—parasitism on a parasite. The term is not limited to fungi and may be used for any group of organisms. Different cases of mycophagy including mycoparasitism may belong to this category. For example, when the host of a mycoparasitic fungus is a plant pathogen, mycoparasite may be consid-

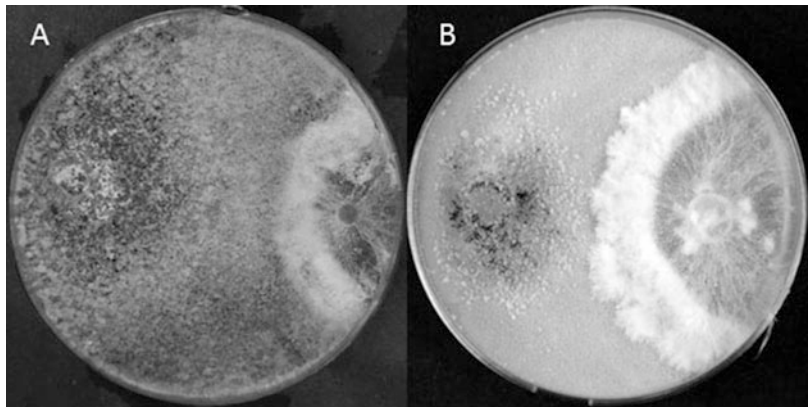

Fig. 12.2 Nutritive and nonnutritive interactions between fungi. (**a**) Mycoparasitism of *Trichoderma harzianum* (*left*) on *Athelia rolfsii*. (**b**) Agonism between *T. reesei* and *A. rolfsii*. *Trichoderma* (*left*) is overgrown by aerial hyphae of *A. rolfsii* (*right*) that does not parasitize on *Trichoderma*

ered as hyperparasite. It is important to note that for any hyperparasitic interactions at least two hosts and two parasites should be present. The use of this term in the absence of the primary host is not correct. For example, *Trichoderma* may be considered as a hyperparasite when it grows on sclerotia of *Athelia rolfsii* (Agaricales, Basidiomycota) that are formed on tomato plants. In this case, both *Trichoderma* and *Athelia* are parasites (and pathogens), respectively, while *Athelia* and tomato are the hosts, respectively. When the same *Trichoderma* is in vitro confronted with the same *Athelia* and it is performed in the absence of tomato, the term pathogen (or parasite) is applicable to *Trichoderma*, not to *Athelia*, and no organisms may be called as a hyperparasite.

- **Antagonism**—a type of **nonnutritive interactions** where one fungus inhibits the growth of other fungi, while continuing to grow uninhibited itself. Similar interactions include **coantagonism** with negative outcome for both fungi and **agonism** when one fungus is harmed and the other receives benefit. The latter interaction is similar to mycoparasitism, but it should not be confused with it as the benefitting fungus does not feed on the one that is harmed. An example for such interaction is *A. rolfsii* that is capable of overgrowing some strains of *Trichoderma* but does not feed on them. The benefit for *A. rolfsii* from this behavior is the reduced competition pressure for space and resources (Fig. 12.2). Tuininga (2005) notes that the term "coantagonism" is preferable to the frequently used term "competition," because the latter term describes only one possible mechanism of antagonistic interactions.

Other theoretically possible and nonnutritive interfungal interactions are bilaterally neutral **cohabitation**, neutral/beneficial **commensalism**, and **mutualism**, but they are very rare in fungi (vide supra) because of the usually present antagonism.

High fungi from many taxonomic groups that are able to either parasitize on plant pathogenic fungi or to antagonize them have been proposed for use in plant protection. For example, in the late 1970s, *Teratosperma sclerotivorum* (syn. *Sporidesmium sclerotivorum*, Ascomycota) that is an obligate pathogen on sclerotia of *Sclerotinia* spp. (Helotiales, Ascomycota) was suggested for use in biological control of the latter plant pathogen (see Fravel 2006 for the review). However, this technology likely did not get commercialized as the recent literature on the topic is limited: public databases contain no gene sequences for this hyperparasitic fungus (NCBI, November 22, 2015), and there is also no recent descriptions of the mechanisms of respective mycoparasitic interactions. Most of the modern antifungal biocontrol formulations use mycotrophic fungi from the order Hypocreales (Sordariomycetes, Pezizomycotina, Dikarya) that are also best studied at molecular biological, ecological, and taxonomic levels, respectively.

IV. Mycotrophic Hypocrealean Fungi

The order Hypocreales from the class of Sordariomycetes contains the best studied mycoparasitic fungi such as *Trichoderma*, *Escovopsis*, and *Clonostachys*, and genome sequences have been obtained for at least one but often several of their species (Fravel 2006; Gruber et al. 2011; Karlsson et al. 2015; Kubicek et al. 2011; de Man et al. 2015; Martinez et al. 2008; Studholme et al. 2013; Xie et al. 2014). Fungi from this order show widely diverse symbiotic associations with plants, animals, and other fungi and are also capable to saprotrophic growth (Sung et al. 2008). The most common animal hosts for hypocrealean fungi are the arthropods from the orders Coleoptera, Hemiptera, and Lepidoptera (Kobayasi 1941; Mains 1958; Sung et al. 2008). Respective arthropod pathogenic hypocrealean fungi consist of several genera mainly from three families: Clavicipitaceae, Cordycipitaceae, and Ophiocordycipitaceae. Nectriaceae and Bionectriaceae mainly feed on plants (Sung et al. 2007, 2008). Although the latter authors marked the family Hypocreaceae as a mixture of fungicolous and plant-associated fungi, recent studies suggest that it is dominated by mycotrophs, of which many taxa may also grow in the rhizosphere or become endophytes (Druzhinina et al. 2011).

Sung et al. (2008) reported and described fossils of the ancient *Paleoophiocordyceps coccophagus*, a fungus belonging to the genus *Ophiocordyceps*, which represents the eldest evidence of animal parasitism by a fungus. This finding allows an estimation of the divergence times of major lineages of Hypocreales which revealed that the hypocrealean fungi were at least present since the Early Jurassic, i.e.; 193 Mya. The authors proposed that the ancestral nutritional state of hypocrealean fungi was plant based, followed by shifts first to animal and then to fungal hosts (Sung et al. 2008). According to this study, the evolution of fungal–animal symbioses of the hypocrealean fungi is characterized by the origin and diversification of three families, Clavicipitaceae, Cordycipitaceae, and Ophiocordycipitaceae, that happened 173 or 158 Mya. The family Hypocreaceae that includes such mycotrophic genera as *Trichoderma* and *Hypomyces* is inferred to have arisen at least 145 Mya (Sung et al. 2008). Their analysis also showed that shifts to fungicolous nutrition occurred several times during the evolution of hypocrealean fungi. It is likely that mycoparasitic *Clonostachys* (Bionectriaceae) that are closely related to plant pathogenic *Fusarium* (Nectriaceae) obtained this possibility diverging from a plant-feeding host, while ancestors of *Trichoderma*, *Verticillium*, and *Escovopsis* likely evolved from animal pathogens. This is nicely illustrated by species of *Elaphocordyceps* (anamorph *Tolypocladium*, Ophiocordycipitaceae) that are mostly parasites of the ectomycorrhizal truffle genus *Elaphomyces* (Eurotiales, Ascomycota), but their next phylogenetic neighbors are all pathogens of insects.

A. *Escovopsis*: The Devastating Pest in Gardens of Leaf-Cutting Ants

The mycoparasitic hypocrealean genus *Escovopsis* is isolated from the nests of fungi-growing leaf-cutting ants, which belong to the tribe Attini (Hymenoptera, Insecta), namely, leaf-cutting ants (*Atta* and *Acromyrmex*), that share an obligate mutualism with Lepiotaceaous fungi of the genus *Leucoagaricus* such as *L. weberi* and *L. gongylophorus* (Agaricales, Basidiomycota) (Currie et al. 2006; Muchovej and Della Lucia 1990) or with pterulaceous fungi (Chapela et al. 1994; Villesen et al. 2004). These fungal cultivars have been acquired by the ants for their gardens from the environment multiple times in the course of evolution (Aylward et al. 2012; Chapela et al. 1994; Mikheyev et al. 2010). The basidiomycetes thereby form specialized hyphae called gongylidia, which serves as the main food supply for the ants (Seifert et al. 1995). In return, the ants provide the fungus with substrate for growth, means of dispersal to new locations, and protection from competitors and parasites (Muchovej and Della Lucia 1990). *Atta* colonies are one of the predominant herbivores in the Neotropics and therefore are frequently considered important agricultural pests in these areas (Hölldobler and Wilson 1990; Wallace et al. 2014). Colonies of these ants exhibit a rapid growth rate, consume hundreds of kilograms of leaves per year (Wirth et al. 2002), and cause the destruction of plantations and gardens in tropical areas of Central and South America and Costa Rica (Reynolds and Currie 2004; Wallace et al. 2014). *Escovopsis weberi* was isolated from nests of leaf-cutting ants as a natural pathogen

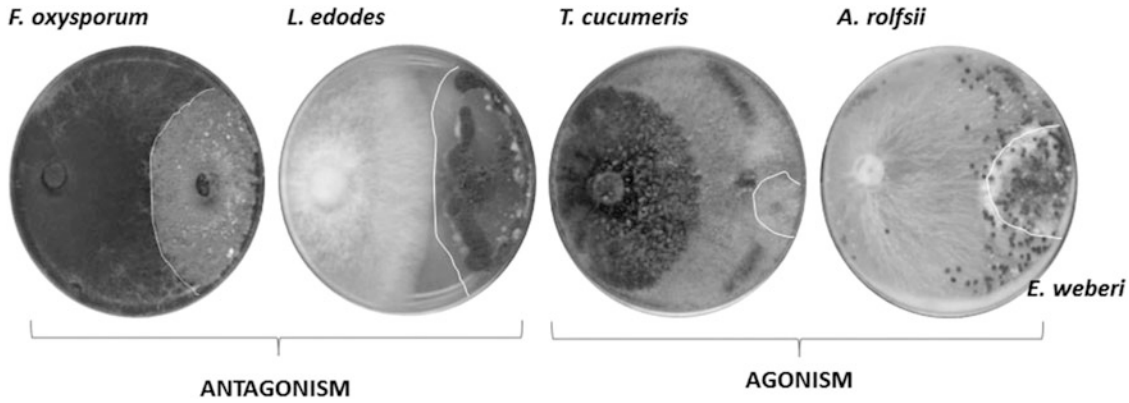

Fig. 12.3 Dual confrontations of *Escovopsis weberi* (*left*) with other fungi. The *white line* indicates the growth of *E. weberi* as detectable from the back side of the plate

of *Leucoagaricus* and was also proposed as a potential bioeffector against these ants (Reynolds and Currie 2004). According to the above explained terminology, *E. weberi* should not be assigned as a hyperparasite because *Leucoagaricus*—its host—is not a parasite but a saprotroph.

Until recently it remained unclear whether the primary nutrient source for *E. weberi* was the mushroom itself or the vegetative substrate placed on the gardens by ants, in other words whether the interaction was nutritive or rather nonnutritive. Reynolds and Currie (2004) demonstrated the true mycoparasitic nature of *E. weberi* by showing its rapid growth on pure culture of *Leucoagaricus* and negligible development on sterilized leaf fragments. Consequently, these authors described *E. weberi* as a necrotrophic mycoparasite of *Leucoagaricus* (Reynolds and Currie 2004). More recently, Marfetán et al. (2015) based on the microscopic analysis of interactions between *E. weberi* and *Leucoagaricus* spp. revealed hooklike structures and the penetration of the host hyphae and thus described *E. weberi* as a true mycoparasite. Furthermore, the most virulent *E. weberi* isolates were those which developed hooks involved in capturing *Leucoagaricus* sp. (Marfetán et al. 2015). The formation of these structures and growth rates positively correlated with virulence of individual *E. weberi* isolates, while the formation of hyphal traps did not show any correlation with virulence. Traps formed by *E. weberi* were also not able to generate pressure over their target nor degrade the *Leucoagaricus* sp. hyphae (Marfetán et al. 2015). Mycoparasitism of *E. weberi* is accompanied by secretion of enzymes and chemotropism toward *Leucoagaricus* (Marfetán et al. 2015; Reynolds and Currie 2004). Moreover water-soluble metabolites secreted by the latter fungus stimulate growth of *E. weberi* and induce its conidiation (Marfetán et al. 2015). Our own results suggest that parasitism of *E. weberi* is specialized on the attack of *Leucoagaricus* spp. (K. Chenthamara and I.S. Druzhinina, unpublished data) We investigated the antifungal potential of *E. weberi* in dual confrontation assays with a standard range of plant pathogenic fungi that are used to estimate the biocontrol potential of *Trichoderma* and *Clonostachys* (Fig. 12.3). We thereby found that *E. weberi* is generally not an aggressive fungus as it is hardly able to attack *Fusarium oxysporum* (Hypocreales, Ascomycota) and is completely agonized by *Thanatephorus* sp. (*Rhizoctonia solani*, Cantharellales, Basidiomycota) and *A. rolfsii* (Agaricales, Basidiomycota). The interaction of *E. weberi* with wood-rotting fungus *Lentinula edodes* (shiitake, Agaricales, Basidiomycota) was more complex as growth of the latter one was somewhat stimulated by the presence of *E. weberi* (data not shown). Our attempts to cultivate *E. weberi* on plates that were pre-colonized by such fungi as *Trichoderma atroviride*, *Alternaria alternata* (Pleosporales, Ascomycota), *A. rolfsii*, and *L. edodes* failed, which suggested that *E. weberi* is not able to parasitize on them (data not shown).

Despite becoming a model system for the study of coevolution and host–parasite dynamics (Currie et al. 2003, 2006; Gerardo et al. 2004; Little and Currie 2008; Mendes et al. 2012; Reynolds and Currie 2004; Rodrigues et al. 2008; Seifert et al. 1995; Taerum et al. 2007, 2010), little attention has been paid to the taxonomy of *Escovopsis* until recently. In the 1990s, when the genus *Escovopsis* was proposed, only two species were known: *E. weberi* (Muchovej and Della Lucia 1990) and *E. aspergilloides* (Seifert et al. 1995). In 2013, three additional *Escovopsis* species—*E. microspora*, *E. moelleri*, and *E. lentecres-*

cens—were described, and a new genus, *Escovopsioides*, was proposed (Augustin et al. 2013). Later on, Meirelles et al. (2015) performed a survey for *Escovopsis* species in gardens of the lower attine ant *Mycetophylax morschi* in Brazil and found four strains belonging to the pink-colored *Escovopsis* clade. The examination of these strains revealed significant morphological differences when compared to previously described species of *Escovopsis* and related *Escovopsioides*. Based on sympodial type of conidiogenesis, percurrent morphology of conidiogenous cells and non-vesiculated conidiophores, Meirelles et al. (2015) described the four new strains as a new species *E. kreiselii*. Phylogenetic analyses using three nuclear markers (28S and ITS1 and 2 or the rRNA operon and the partial sequence of the translation elongation factor 1-alpha, *tef1*) from the new strains and sequences retrieved from public databases confirmed that all known fungi infecting attine ant gardens comprise a monophyletic group within the Hypocreaceae family. Specifically, *E. kreiselii* is likely associated with gardens of lower attine ants, but the mode of its pathogenicity remains uncertain. Even more interestingly, a further new species of *Escovopsis*, *E. trichodermoides*, isolated from a fungus garden of the lower attine ant *Mycocepurus goeldii*, which has highly branched, *Trichoderma*-like conidiophores lacking swollen vesicles, with reduced conidiogenous cells and distinctive conidia morphology, was described by Masiulionis et al. (2015). We compared *tef1* sequences of the two almost simultaneously described and therefore not compared *Escovopsis* species and found that they are only 90 % similar (see NCBI accession numbers KF033128 and KJ808766 for *E. trichodermoides* and *E. kreiselii*, respectively). Thus, in November 2015, there are seven species of *Escovopsis* recorded in the Index Fungorum database (http://www.indexfungorum.org/): *E. aspergilloides*, *E. kreiselii*, *E. lentecrescens*, *E. microspore*, *E. moelleri*, *E. trichodermoides*, and the oldest *E. weberi*. All these taxa are only known from gardens of leaf-cutting ants.

The genome of *E. weberi* was sequenced by de Man et al. (2015) and shown to have a significantly reduced size and gene content compared to closely related but less specialized mycotrophic fungi from the genus *Trichoderma* (Kubicek et al. 2011; Martinez et al. 2008), which emphasizes the specialized nature of the interaction between *Escovopsis* and ant agriculture. While genes for primary metabolism have been retained, the *E. weberi* genome is depleted in carbohydrate-active enzymes, which may represent a reliance on a host capable to perform these functions. *E. weberi* has also lost genes necessary for sexual reproduction. Contrasting these losses, the genome encodes unique secondary metabolite biosynthesis clusters, some of which exhibit upregulated expression during host attack. The availability of the whole genome sequences of *E. weberi* and several species of *Trichoderma* makes the detailed comparison of ecophysiology of these fungi a challenging task.

B. Versatile Mycoparasites from the Genus *Trichoderma*

Of all mycoparasites and/or mycotrophs, the hypocrealean genus *Trichoderma* is probably the best studied and the most frequently applied bioeffector with the widest host/prey range (Atanasova et al. 2013; Baek et al. 1999; Brunner et al. 2005; Druzhinina et al. 2011; Elad et al. 1980; Kotasthane et al. 2015; Kubicek et al. 2011; Mukherjee et al. 2013; Studholme et al. 2013; Zhang et al. 2015). One of the many important qualities that makes *Trichoderma* outstanding as a biological control agent for plant pathogenic fungi (biocontrol; see below) is its high opportunistic potential (Jaklitsch 2011; Jaklitsch 2009) and adaptability to various ecological niches (Atanasova 2014). It has been well documented that *Trichoderma* spp. used for biocontrol can act through a diversity of mechanisms and combinations of them. Despite of the fact that these fungi are mycoparasites, necrotrophic mycoparasites, and nonspecific mycotrophs (Kubicek et al. 2011; see also Druzhinina and Kubicek 2013, for more references), they can establish themselves in the rhizosphere and stimulate plant growth and thus elicit a general plant defense reactions against pathogens (Druzhinina et al. 2011; Galletti et al. 2015; Harman 2011; Kotasthane et al. 2015). Some *Trichoderma* spp. have been also

isolated as endophytes too (Bae et al. 2009; Bongiorno et al. 2015; Chaverri et al. 2015; Gazis and Chaverri 2010; Rosmana et al. 2015). All of these characteristics make *Trichoderma* a genus of particular interest for application in agriculture as biofungicide and biofertilizer.

The genomic properties of *Trichoderma* spp. that add to their ability for biocontrol have been discussed (Martinez et al. 2008; Kubicek et al. 2011). In general these properties can be divided into such related to interactions with other fungi (Fig. 12.4) and such related to the interactions with plants and nonfungal pathogens of plants (nematodes, bacteria). As the latter topic is behind the scope of this review, the following description will only consider interfungal interactions with participation of *Trichoderma*. Druzhinina et al. (2011) and Druzhinina and Kubicek (2013) provided detailed reviews of *Trichoderma*'s ability to interact with living fungi as both mycoparasites and predators (necrotrophic mycoparasites) and also to their ability to saprotrophically feed on dead fungal biomass. The targeted biotrophic interaction of *Trichoderma* with other fungi includes such steps as sensing the presence of the host and optional coiling around their hyphae. host cell wall degradation and penetration of the host hyphae, repair of damages caused by hosts, and production of toxic secondary metabolites that may eventually kill the host and thus transforming it to a prey. In this chapter we will focus on those studies that functionally characterized genes involved in the interactions between *Trichoderma* and other fungi (Table 12.1). Most of them are involved in signal transduction during mycoparasitism, in fungal cell wall degradation, and in the production of antifungal secondary metabolites. Fewer studies focused on general and specific regulator genes such as *nox1*, *noxR*, *laeA*, *vel1*, and *xyr1* and the role of proteases.

Table 12.1 demonstrates that the absolute majority of functional genetic investigations were performed on two species of *Trichoderma* only, i.e. *T. atroviride* and *T. virens*. Atanasova et al. (2013) used DNA microarrays to compare the transcriptional response of the latter two species in comparison to *T. reesei* to the presence of *Thanatephorus cucumeris* (*Rhizoctonia solani*). They found that the three *Trichoderma* spp. exhibited a strikingly different transcriptomic response already before physical contact with alien hyphae. *T. atroviride* expressed an array of genes involved in the production of secondary metabolites, GH16 β-glucanases, various proteases, and small secreted cysteine-rich proteins. *T. virens*, on the other hand, expressed mainly the genes for biosynthesis of gliotoxin, respective precursors, and also glutathione, which is necessary for gliotoxin biosynthesis. In contrast, *T. reesei* increased the expression of genes encoding cellulases and hemicellulases and of the genes involved in solute transport. The majority of differentially regulated genes were orthologs present in all three species or both in *T. atroviride* and *T. virens*, indicating that the regulation of expression of these genes is different in the three *Trichoderma* spp. The genes expressed in all three fungi exhibited a nonrandom genomic distribution, indicating a possibility for their regulation via chromatin modification. The authors concluded that the initial *Trichoderma* mycotrophy demonstrated earlier by Kubicek et al. (2011) has differentiated into several alternative ecological strategies. In the context of their study, when *T. cucumeris* was used as an opponent for *Trichoderma*, the interactions ranged from parasitism of *T. atroviride* to predation of *T. virens* and competitive cohabitation of *T. reesei*. The neutral response of the latter species is best explained by the fact that the exclusively tropical *T. reesei* has never been isolated from soil so far and is not able to recognize temperate soil-borne *T. cucumeris* as its host or prey (Druzhinina et al. 2010). But it is important to note here that the assumption that *T. reesei* is merely a saprotrophic fungus that is not capable to mycotrophy is contradicted by numerous studies that demonstrated the ability of this fungus to attack a variety of fungi (Druzhinina et al. 2010; Atanasova et al. 2013), as also shown in Fig. 12.4.

The other conclusion that can be drawn from Table 12.1 is that the majority of the studies made were based on only a limited number of opponent fungi. In most studies either *T. cucumeris*, *A. rolfsii*, or *Botrytis cinerea* (Helotiales, Ascomycota) was used for confrontations with *Trichoderma*. As most *Trichoderma* species are capable of biotrophic and necrotrophic types of mycoparasitism and may also efficiently feed on dead fungal biomass, the conclusions of these studies therefore demonstrated only partial reduction of either one or another mycotrophic strategy employed by the respective *Trichoderma* species in given interactions. The

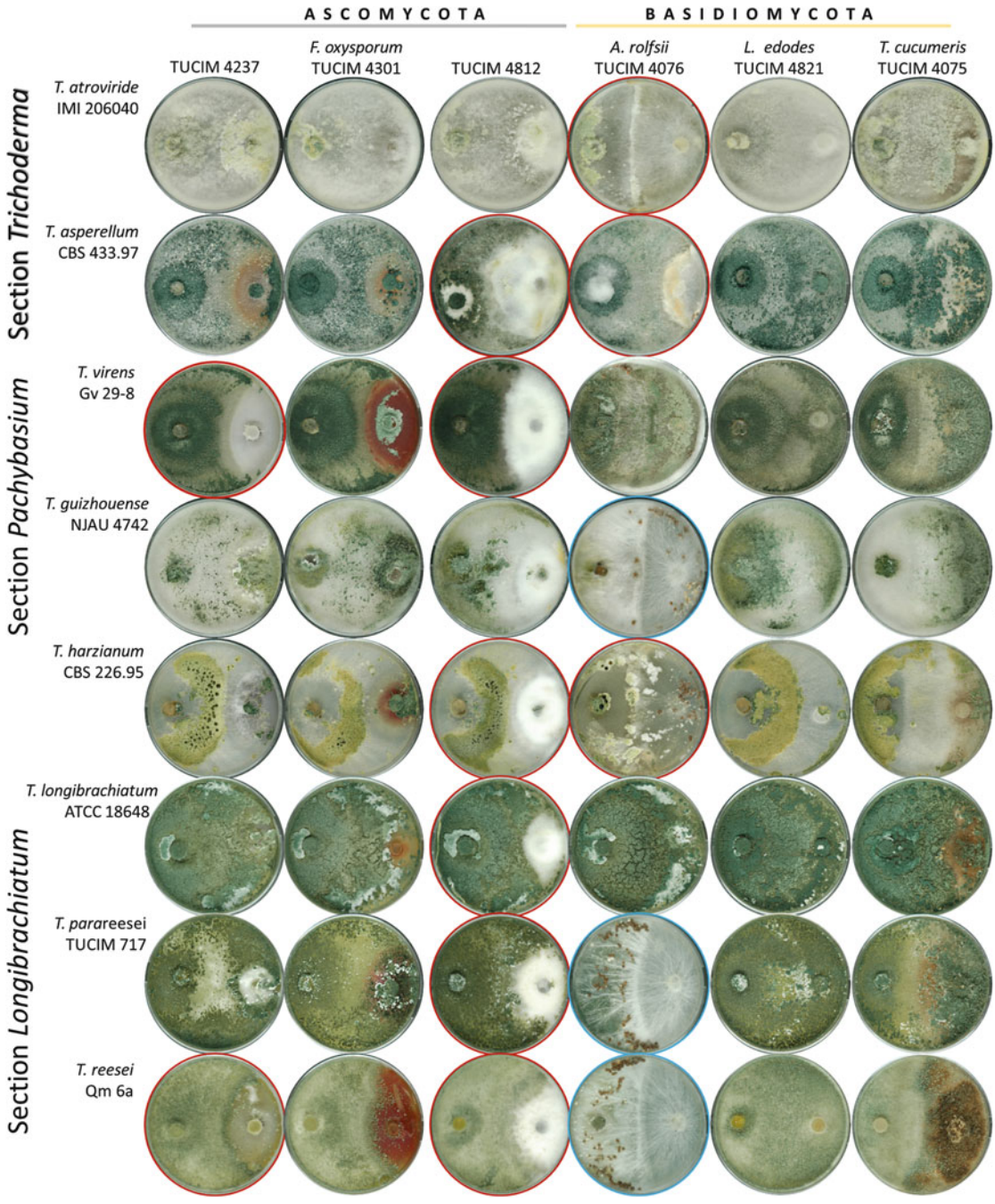

Fig. 12.4 In vitro interactions between *Trichoderma* (*left*) and other fungi in dual confrontation assays as observed after 10 days of incubation on PDA medium at 25° and 12 h cyclic illumination. Cases of non-mycoparasitic interactions are marked by *dark red* (antagonism) and *blue* (agonism) background

recent study by Zhang et al. (2015) demonstrated a clear role of *nmp1* gene encoding a secreted neutral deuterolysin metallopeptidase in the predation by *T. guizhouense* [former *T. harzianum* species complex (Chaverri et al. 2015; Li et al. 2012)] on *F. oxysporum*, *A. rolfsii*, and *A. alternata*. However, NMP1 was also found to be involved in mycoparasitism on *B. cinerea* and *S. sclerotiorum* and did not have any role in the

Table 12.1 Genes of *Trichoderma* that have been studied for their role in fungal–fungal interactions

Mycoparasitism-related process	Gene	General function	Predator/parasite	Prey/host	Phenotype due to the deletion or silencing	Phenotype caused by the gene overexpression	Study	Year
Signal transduction	*tga1*	G-protein α-subunit; takes part in G-protein-mediated signaling pathway; involved in conidiation, sexual reproduction; important for sensing mating partners/preys/hosts	*T. atroviride*	*Thanatephorus cucumeris* (*Rhizoctonia solani*)	Light-independent hyper-sporulation; retarded mycoparasitic-related coiling against *R. solani*; loss of GTPase activity, which is stimulated by the peptide toxin, Mas-7	An increase of coiling; inhibited sporulation; increased mycoparasitism on *R. solani*	Reithner et al.	2005
Signal transduction	*tga3*	G-protein α-subunit; takes part in G-protein-mediated signaling pathway; involved in conidiation, sexual reproduction; important for sensing mating partners/preys/hosts	*T. atroviride*	*T. cucumeris* (*R. solani*); *Botrytis cinerea*	Reduced growth; defect in chitinase secretion; loss of infection structure formation; avirulence against *R. solani* and *Botrytis cinerea*	n.a.	Zeilinger et al.	2005
Signal transduction	*tac1*	Adenylate cyclase, a signal regulator in cAMP signaling pathway	*T. virens*	*Athelia rolfsii* (*Sclerotium rolfsii*); *T. cucumeris* (*R. solani*); *Pythium* sp.[a]	Retarded morphology; retained only 5–6 % of the wild-type growth rate on agar; lowered intracellular cAMP levels below the detection limit; loss of virulence against *Athelia rolfsii*, *R. solani*, and *Pythium* sp.; negatively affected production of secondary metabolites	n.a.	Mukherjee et al.	2007
Signal transduction	*tmkA*	Mitogen-activated protein kinase (MAPK) signaling pathway gene, involved in transmitting signals for mating, filamentous growth, cell integrity, response to osmotic stress, and ascospore formation	*T. virens*, *T. atroviride*	*A. rolfsii* (*S. rolfsii*); *T. cucumeris* (*R. solani*)	Reduced ability to parasitize *R. solani* and *S. rolfsii* and the ability to induce systemic resistance in plant; in another strain of *T. virens*, improved mycoparasitism against *R. solani*; in *T. atroviride*, it resulted in improved mycoparasitism	n.a.	Mukherjee et al. Viterbo et al. Mendoza-Mendoza et al. Reithner et al.	2003 2005 2003 2007
Signal transduction	*tmkB*	Mitogen-activated protein kinase (MAPK) signaling pathway gene, involved in transmitting signals for mating, filamentous growth, cell integrity, response to osmotic stress, and ascospore formation	*T. virens*	*A. rolfsii* (*S. rolfsii*); *T. cucumeris* (*R. solani*); *Pythium* sp.[a]	Reduced radial growth; constitutive conidiation in darkness; defects in cell wall integrity (autolysis of the mycelia and increased sensitivity to cell wall-degrading enzymes); attenuated ability to overgrow the plant pathogen *A. rolfsii*	n.a.	Kumar et al.	2010
Signal transduction	*hog1*	High-osmolarity glycerol response, along with other proteins is required to develop stress resistance	*T. harzianum*	*Phoma betae* (Pleosporales, Ascomycota); *Colletotrichum acutatum* (Glomerellales, Ascomycota)	Reduced osmotic and oxidative stress tolerance; reduced antagonistic activity against the tested plant pathogens	n.a.	Delgado-Jarana	2006

(continued)

Table 12.1 (continued)

Mycoparasitism-related process	Gene	General function	Predator/parasite	Prey/host	Phenotype due to the deletion or silencing	Phenotype caused by the gene overexpression	Study	Year
Cell wall degradation	*ech42* (*chi18-5*)	Endochitinase, secreted	*T. atroviride*, *T. harzianum*, *T. virens*	*A. rolfsii* (*S. rolfsii*); *T. cucumeris* (*R. solani*)	In *T. harzianum*: unaffected mycoparasitism of *A. rolfsii* and *T. cucumeris*. In *T. virens*: reduced mycoparasitism of *T. cucumeris*	*T. atroviride*: improved mycoparasitism	Carsolio et al. Deng et al. Baek et al.	1999 2007 1999
Cell wall degradation	*nag1*	*N*-acetyl-β-D-glucosaminidase, secreted	*T. atroviride*, *T. harzianum*	*T. cucumeris* (*R. solani*); *B. cinerea*	In *T. atroviride*: resulted in reduced ability to protect bean seedlings against *R. solani*. In *T. harzianum*: resulted in reduced antagonistic activity against *B. cinerea*	n.a.	Brunner et al. Dubey et al.	2003 2012
Cell wall degradation	*bgn3*	β-1,6-glucanase, secreted	*T. virens*	*Rhizopus oryzae* (Mucorales, Mucoromycotina); *T. cucumeris* (*R. solani*); *P. ultimum*[a]	No effect on growth and development; reduced ability to inhibit growth of *P. ultimum*	Improved antagonism against *P. ultimum*, *Rhizopus oryzae*, and *T. cucumeris*	Djonović et al.	2006b
Cell wall degradation	*thpg1*	Endopolygalacturonase, secreted	*T. harzianum*	*T. cucumeris* (*R. solani*); *B. cinerea*; *P. ultimum*[a]	Reduced PG activity, ability to grow on pectin medium, and ability to colonize *Solanum lycopersicum* (tomato) (Solanales, Streptophyta) roots	n.a.	Morán-Diez et al.	2009
Regulator	*nox1*	NADPH oxidase	*T. atroviride*, *T. harzianum*	*P. ultimum*[a]	Severely affected ability to form conidia in response to injury; uncompromised hyphal regeneration; loss in ability to produce reactive oxygen species (ROS) in response to injury	In *T. harzianum*: resulted in improved biocontrol potential against *P. ultimum*	Montero-Barrientos et al. Hernández-Oñate et al.	2011 2012
Regulator	noxR	Regulator of NADPH oxidases involved in reactive oxygen species formation	*T. atroviride*	n.a.	Affected ability to form conidia in response to injury; hyphal regeneration was not compromised	n.a.	Hernández-Oñate et al.	2012
Regulator	*laeA*	Methyltransferase. Global regulator that affects the expression of secondary metabolite gene clusters and controls sexual and asexual development	*T. atroviride*	*Alternaria alternata*; *T. cucumeris* (*R. solani*); *B. cinerea*	Decrease in conidiation by 50 % in light; no conidiation in darkness; abolishment of sporulation in response to injury; increased sensitivity to oxidative stress; affected expression of genes encoding several proteases, GH16 β-glucanases, PKSes, and SSCPs; decrease in antagonism against *A. alternata*, *T. cucumeris*, and *B. cinerea*; decrease in production of known antifungal metabolites including 6PP (6-pentyl-2H-pyran-2-one)	Increased conidiation by 30–50 % in light; enhanced mycoparasitic vigor; resistance to oxidative stress (H_2O_2, 5 mM); increased production of a known antifungal metabolite, 6PP (6-pentyl-2H-pyran-2-one)	Aghcheh et al.	2013

General regulator	*xyr1*	Regulator protein for cellulase and hemicellulase gene expression in *Trichoderma*	*T. atroviride*	*Phytophthora capsici* (Peronosporales)[a]; *B. cinerea*; *T. cucumeris* (*R. solani*)	Reduced transcript levels of *axe1* and *swo1*, which encode accessory cell wall-degrading enzymes; delayed response of *Arabidopsis thaliana* during *Trichoderma–Arabidopsis* interaction; upregulation of *prb1* expression; overall enhanced competition with studied plant pathogens probably due to overexpression of *prb1*	n.a.	Reithner et al.	2014
Secondary metabolites	*tri4*	Cytochrome P450 monooxygenase that oxygenates trichodiene to give rise to isotrichodiol	*T. arundinaceum*, *T. harzianum*	*B. cinerea*; *T. cucumeris* (*R. solani*)	In *T. arundinaceum*: reduced antifungal activity against *B. cinerea* and *T. cucumeris*; reduced ability to induce the expression of *S. lycopersicum* defense-related genes belonging to the salicylic acid (SA) and jasmonate (JA) when attacked by *B. cinerea*	In *T. harzianum*: reduced ability to colonize roots; downregulation of defense-related genes in *S. lycopersicum*	Malmierca et al. Cardoza et al.	2012 2015
Secondary metabolites	*tri5*	Terpene synthase	*T. arundinaceum*, *T. brevicompactum*	*B. cinerea*, *T. cucumeris* (*R. solani*)	In *T. arundinaceum*: no production of HA (a non-phytotoxic trichothecene), which has role in antagonistic activity against fungal plant pathogens and induction of plant genes involved in defense responses; altered the expression of other tri genes involved in HA biosynthesis; altered the expression of hmgR, dpp1, erg9, erg1, and erg7, all genes involved in terpene biosynthetic pathways	In *T. brevicompactum*: increase of the trichodermin; increase in the antibiotic activity against a large panel of yeasts; affected *S. lycopersicum* growth and the lesions caused by *B. cinerea*	Malmierca et al. Tijerino et al.	2013 2011
Secondary metabolites	*gliP*	Involved in the production of gliotoxin, one of the most studied secondary metabolite in fungi that is known for its strong antimicrobial and cytotoxic activity. It is also successfully investigated as an antibiotic	*T. virens*	*Sclerotinia sclerotiorum*; *T. cucumeris* (*R. solani*); *P. ultimum*[a]; *Galleria mellonella* (Lepidoptera, Arthropoda)[b]	Abolition of gliotoxin production; reduced growth; dispersed and less dense mycelium; less branched hyphae; increased sensitivity to oxidative stress (H_2O_2, 10 mM); ineffective as mycoparasites against *P. ultimum*, *S. sclerotiorum*, but retained mycoparasitic ability against *T. cucumeris*. Reduced entomopathogenic activity against *G. mellonella*	n.a.	Vargas et al.	2014

(continued)

Table 12.1 (continued)

Mycoparasitism-related process	Gene	General function	Predator/parasite	Prey/host	Phenotype due to the deletion or silencing	Phenotype caused by the gene overexpression	Study	Year
Regulator	*vel1*	Velum formation protein 1; known to be one of the regulators of morphogenesis and secondary metabolism in some filamentous fungi	*T. virens*	*R. solani*, *P. ultimum*[a]	Defective gliotoxin production; no conidiation; early chlamydospore formation under nutrient stress conditions; delayed or eliminated chlamydospore formation in nutrient-rich media; absence of mycelial and extracellular pigments; defects in the regulation of many other secondary metabolism-related genes; decrease in mycoparasitism against *T. cucumeris* and *P. ultimum*	n.a.	Mukherjee and Kenerley	2010
Secondary metabolites	*pks4*	Polyketide synthase 4, involved in the production of the characteristic green pigment and the non-melanized structures of fruiting bodies in *Trichoderma*	*T. reesei*	*R. solani*; *S. sclerotiorum*; *A. alternate*	Loss of green pigmentation in their conidia; reduced resistance to UV; reduced stability of the conidial wall and the antagonistic abilities against *R. solani*, *S. sclerotiorum*, and *A. alternate*; reduced formation of water-soluble antifungal metabolites; altered expression of other PKS-encoding genes	n.a.	Atanasova et al.	2013
Proteases	*prb1*	Alkaline serine protease, secreted	*T. atroviride*[c]	*T. cucumeris* (*R. solani*)	n.a.	Improved mycoparasitism	Flores et al.	1997
Protease	*sp1*	Serine protease, secreted	*T. virens*	*T. cucumeris* (*R. solani*)	No effect on growth rate, conidiation, extracellular protein accumulation, antibiotic profiles, or the ability to induce phytoalexins in *Gossypium* (Malvales, Streptophyta) seedlings	Increase the ability of to protect *Gossypium* seedlings	Pozo et al.	2004
Protease	*nmp1*	Neutral deuterolysin metallopeptidase, secreted	*T. guizhouense*	*A. rolfsii*; *T. cucumeris*; *B. cinerea*; *S. sclerotiorum*; *A. alternata*; *Fusarium oxysporum*; *F. fujikuroi*	Reduced mycoparasitism; no coiling around hyphae of *F. oxysporum* f. sp. *cubense* 4; reduced ability to produce antifungal secondary metabolites; reduced ability to defend against other fungi	Increased mycoparasitism; self-toxicity	Zhang et al.	2015

Accessory proteins	*sm1*	Cerato-platanin, SSCPs	*T. virens*	n.a.	Unaffected growth or development, conidial germination, production of gliotoxin, hyphal coiling, hydrophobicity, or the ability to colonize *Zea mays* roots; same levels of systemic protection as in plants that have not been treated with *Trichoderma*; reduced level of protection in plants against diseases (*Colletotrichum graminicola* was used as a plant pathogen)	Unaffected growth or development, conidial germination, production of gliotoxin, hyphal coiling, hydrophobicity, or the ability to colonize *Z. mays* roots; enhanced levels of protection against plant diseases	Djonović et al. Djonović et al. (2006b) Salas-Marina et al.	2007 2006 2015
Accessory proteins	*sm2*	Cerato-platanin, SSCPs	*T. virens*	n.a.	Dramatic decrease (more in compared to *sm1*) in the ability of the fungus to induce resistance against disease caused by *Cochliobolus heterostrophus* in *Zea mays* (maize) (Poales, Streptophyta)	n.a.	Gaderer et al.	2015
Accessory proteins	*epl1*	Cerato-platanin, SSCPs	*T. atroviride*, *T. virens*	*Alternaria solani*; *B. cinerea*	In *T. atroviride*: resulted in diminished systemic protection of tomato plants (*S. lycopersicum*) against *A. solani* and *B. cinerea*, whereas in *T. virens* was less effective in protecting tomato against *Pseudomonas syringae* pv. tomato (Pseudomonadales, Proteobacteria) and *B. cinerea*	An increase in disease resistance against all tested pathogens	Salas-Marina et al.	2015

[a, b]Indicate nonfungal hosts from Oomycota and Insecta, respectively

[c]The strain IMI 206040 was initially published as *T. harzianum*

efficient attack of this fungus on *T. cucumeris* at all. Moreover, the secretion of the protein was induced when the fungus was confronted with itself on dead fungal biomass as the carbon source and was not activated when *T. guizhouense* was grown on glucose or potato dextrose agar. Besides the role of the exact protease that is definitely only one of the numerous other proteases that likely act synergistically in different *Trichoderma* species (Druzhinina et al. 2012), this study demonstrates the diversity of types of interaction that may be formed by one individual *Trichoderma* strain against a broad range of opponent fungi. It is thus impossible to assign *Trichoderma* to either exclusively biotrophic mycoparasitic fungi or describe them as necrotrophic mycoparasites or saprotrophs. Figure 12.4 illustrates the diversity of interactions between eight *Trichoderma* strains from eight species representing the three major infrageneric clades and six opponent fungi including three closely related strains of *Fusarium oxysporum* and three unrelated Basidiomycota fungi. For this reason, Druzhinina et al. (2011) proposed the more general term mycotroph as the best ecological identifier for *Trichoderma* spp. Results of Zhang et al. (2015) also demonstrate the need to study the role of individual genes in at least several possible interactions including at least parasitism and predation.

C. *Clonostachys rosea* Demonstrates an Alternative Toolkit for Successful Mycoparasitism

The mechanisms of interfungal interactions with the participation of still another hypocrealean mycotrophic fungus—*Clonostachys rosea*—have only recently attracted researchers' interest. Schroers et al. (1999) classified the mycoparasite *Gliocladium roseum* as *Clonostachys rosea* because it differed from the type species of *Gliocladium*, *G. penicillioides*, in morphology, ecology, teleomorph, and DNA sequence data. Jensen et al. (2004) used an ecological approach to present *C. rosea* as an effective mycoparasite against *Alternaria dauci* (Pleosporales, Ascomycota) and *A. radicina* on carrots (*Daucus carota* subsp. *sativus*). *C. rosea* showed a similar efficiency against these pathogens as the fungicide iprodione. A *C. rosea* strain, *C. rosea* IK726, was transformed with GFP (green fluorescent protein) and was used in biopriming of carrot seeds. Microscopy after 7 days of this biopriming showed seeds covered with a fine web of sporulating mycelium of *C. rosea*. Rodríguez et al. (2011) demonstrated the antagonism of *C. rosea* BAFC3874 against *Sclerotinia sclerotiorum* (Helotiales, Ascomycota) in pot-grown lettuce (*Lactuca sativa*) and soybean (*Glycine max*) plants and established that the strain produced antifungal compounds. They comprised a microheterogeneous mixture of peptaibols. These are short linear peptides that are rich in α-aminoisobutyric acid and bear an acetylated N-terminus and an amino alcohol at the C-terminus (Kubicek et al. 2007). They form helices that are inserted into the plasma membrane of the host causing alterations in the osmotic balance of the cell (Degenkolb et al. 2006) and inhibit membrane-bound enzymes such as cell wall polysaccharide synthases (Lorito et al. 1996). Such effects may explain some of the changes observed in the mycelium of the pathogen, including cell lysis of the hyphae and melanization (Rodríguez et al. 2011).

Recently, Karlsson et al. (2015) sequenced the whole genome of *C. rosea* IK726. A comparative phylogenetic analysis between *C. rosea*, *Trichoderma* spp., and *Fusarium* spp. suggested that *C. rosea* are sister taxa to *Fusarium* spp. (frequent plant pathogens), which belongs to family Bionectriaceae. In their study *Trichoderma* spp., which belongs to family Hypocreaceae, appeared in basal position to *C. rosea*. A comparative analysis of gene family evolution under the hypothesis that evolution of mycoparasitism in Bionectriaceae and Hypocreaceae results in selection for converging interaction mechanisms, revealed several differences between the studied mycoparasites. In comparison to *Trichoderma* spp., *C. rosea* showed expansion in several gene families such as those involved in plant cell wall degradation (polysaccharide lyase family 1 (pectin lyase), auxiliary activity family 3 (glucose–methanol–choline oxidoreductases), and auxiliary activity family 9 (lytic polysaccharide monooxygenase)); secondary metabolite synthesis (PKS, cytochrome P450 monooxygenases, PKS, and NRPS genes), likely attributing to production of antifungal components; ABC transporter and major facility superfamily membrane transporters, attributing to the fungi's high tolerance to toxins like boscalid, ZEN, and other microbial metabolites; and several ankyrin repeat proteins. In contrast, the genome of *C. rosea* contains significantly fewer carbohydrate-binding family 18 (CBM18, chitin binding) module containing genes (only two B group GH18 chitinases, only two C group GH18 chitinases, eight A group GH18 chitinases), suggesting that cell wall degradation of the fungal prey may not be a prominent strategy for interactions of *C. rosea* with other fungi.

Table 12.2 summarizes the results of functional characterization of *C. rosea* genes

Table 12.2 Genes of *Clonostachys rosea* studied for their role in fungal–fungal interactions

Mycoparasitism-related process	Genes	General function	Prey/host	Phenotype due to the deletion or silencing	Expression analysis	Study	Year
Secondary metabolites	*zhd101*	Zearalenone hydrolase	*F. graminearum*	Lowered in vitro ability to inhibit growth of the ZEA-producing *F. graminearum*. Failed to protect wheat seedlings against foot rot caused by the ZEA-producing *F. graminearum*	n.a.	Kosawang et al.	2014
Accessory proteins	*hyd1*, *hyd2*, *hyd3*	Hydrophobins	*F. graminearum*; *B. cinerea*; *T. cucumeris* (*R. solani*)	Higher growth rate of *Δhyd1Δhyd3* in high salinity. Faster overgrowth of *Δhyd1Δhyd3*, *Δhyd1*, and *Δhyd3* on prey/hosts. Improved protection of plants against fungal pathogens. Increased colonization ability by *Δhyd1Δhyd3* on *Arabidopsis thaliana* roots	Repressed expression of *hyd1*, *hyd2*, and *hyd3* in interactions with *B. cinerea* and *F. graminearum*. Upregulation of *hyd1*, *hyd2*, and *hyd3* during self-interaction. High expression of *hyd1* in germinating conidia	Dubey et al.	2014
Transporter	*abcG29*	ATP-binding cassette (ABC) transporter, induced by zearalenone and the fungicides Cantus, Chipco Green, and Apron	*F. graminearum*, *F. oxysporum* f. sp. *radicis-lycopersici*; *B. cinerea*	Delay in conidial germination and subsequent reduction in total germ tube length when subjected to H_2O_2; increase in necrotic lesion area caused by *B. cinerea* measured on *A. thaliana* leaves pre-inoculated with *ΔabcG29* strain spores compared to the necrotic lesion area on leaves pre-inoculated with *C. rosea* WT spores; increase of *F. graminearum* foot rot disease severity in barley seedlings	n.a.	Dubey et al.	2015
Transporter	*abcG5*	ATP-binding cassette (ABC) transporter, induced by zearalenone, secondary metabolites secreted by *F. graminearum* and different classes of fungicides	*F. graminearum*	Reduced antagonism toward *F. graminearum*; reduced biocontrol efficiency to protect barley seedlings from foot rot disease caused by *F. graminearum*; decreased tolerance to xenobiotics secreted by *F. graminearum* and toward ZEN; iprodione-and mefenoxam-based fungicides	n.a	Dubey et al. (2014b)	2014b

required for its interaction with other fungi. The availability of the genome sequence and its comparative analysis now provides the basis for studying of those gene families that are overrepresented in the genome of this fungus (*vide supra*).

D. Further Candidates for Whole Genome Sequencing of Mycoparasitic Fungi

A number of other hypocrealean fungi are mycotrophic with different degrees of specialization. However, most of them remain poorly investigated. These are such genera as, for example, *Hypomyces* (Põldmaa et al. 1997), *Cosmospora*, and *Verticillium*, but none of them have been investigated on the levels of genes and/or genomes. *Hypomyces*, with about 50 species recognized in recent studies (Poldmaa 1996; Rogerson and Samuels 1985, 1989, 1993, 1994), among which more than 30 are listed in NCBI taxonomy browser (November 2015), is the largest genus of almost exclusively fungicolous fungi.

Hypomyces species occur mainly on discomycetes, boletes, agarics, or polypores. The polyporicolous *Hypomyces* are more numerous than any other group, with 19 species accepted by Rogerson and Samuels (1993). The genome of *Hypomyces chrysospermus* CBS 394.52, a bolete mold that grows on *Boletus* (Polyporales, Basidiomycota) mushrooms, turning the host a whitish, golden yellow, or tan color and making it not edible, is currently sequenced by JGI DOE in collaboration with Joe Spatafora (https://gold.jgi.doe.gov/project?id=36363). The same group has sequenced the whole genome sequence of *Tolypocladium inflatum* (Bushley et al. 2013), which is a pathogen of beetle larvae but is closely related to fungicolous species of *Elaphocordyceps*. The comparative analysis of *H. chrysospermus* with already sequenced fungicolous hypocrealean fungi and *T. inflatum* may give insights in convergent evolution of this lifestyle.

Sphaerodes quadrangularis (Ceratostomataceae, Hypocreales) is another example of a facultative (i.e., able to grow saprotrophically in vitro, even when the host is absent) contact biotrophic mycoparasite. It establishes an intimate relationship with its host *Fusarium avenaceum* (Nectriaceae, Hypocreales) by producing hook-shaped and clamp-like attachment structures that appeared to derive nutrients and essential growth factors from living host cells (Vujanovic and Goh 2009).

Vujanovic and Goh (2009) demonstrated that *S. quadrangularis* produces hook-shaped structures within four days of subjection with *F. avenaceum*. Although *S. quadrangularis* also produces clamp-like or other contact structures, hook-shaped contact cells are more prominent. It is interesting that the diameter of hyphae parasitizing *F. avenaceum* is much smaller compared to its saprotrophic hyphae. *S. quadrangularis* also shows host specificity, as it did not form any specialized attachment structures when confronted with other *Fusarium* species. *S. mycoparasitica*, another biotrophic mycoparasite from the same genus, has however proven to be effective against a broad range of *Fusarium* species, showing positive effect on wheat (*Triticum* spp.) seed germination and seedlings growth (Vujanovic and Goh 2012). This fungus is not affected by the mycotoxins produced by *F. graminearum* such as deoxynivalenol (DON), trichothecene, and its acetyl derivatives 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (ADON), and zearalenone (ZEA) (Shinha and Bhatnagar 1998), probably because of the presence of similar defense genes as found in *C. rosea* (Karlsson et al. (2015).

An interesting further target for more detailed investigations may be *Calcarisporium arbuscula* (Watson 1955) that is only putatively related to Hypocreales based on the similarity of its nucleotide sequence encoding *rpb2* gene for the RNA polymerase II large subunit 2 (NCBI accession number LN714633). Although the morphology of interaction of these fungi with their hosts that belong to Xylariales (cf. *Physalospora*) has been interpreted by Barnett (1958) as intimate balanced mycoparasitism, no advanced recent studies on this fungus have been performed. The low attention to *Calcarisporium* is demonstrated by only 18 nucleotide sequences deposited in public databases for the entire genus (NCBI, November 2015), none of which were obtained in relation to studies of interfungal interactions.

Numerous other fungicolous fungi are only investigated on the level of their taxonomy that also frequently yields unexpected results. For example, Hawksworth et al. (2010) studied *Roselliniella*, a pyrenocarpous fungi growing on lichens and forming single-celled brown ascospores and persistent interascal filaments that were previously assigned to Sordariales. The molecular phylogeny showed them to belong to Hypocreales. Jaklitsch and Voglmayr (2014) investigated and

reinstated the fungicolous genus *Thyronectria* as also belonging to Hypocreales.

V. First Transcriptomic Insight into Mycoparasitism of *Ampelomyces quisqualis*

Mycoparasitic fungi from other groups than Hypocreales are studied less intensively. One exception is the mycotrophic fungus *Ampelomyces quisqualis* (Pleosporales, Ascomycota) that is a hyperparasite of *Erysiphe*, *Podosphaera*, *Sphaerotheca*, *Uncinula*, and others that all belong to the order Erysiphales (Ascomycota) and cause powdery mildew disease of wine grapes, cucumber, carrots, mango, and other plants (Sztejnberg et al. 1989; Takamatsu 2004). In total *Ampelomyces* has been described to be associated with more than 60 species from eight different genera of the order Erysiphales and is thus the most widespread and oldest known natural enemy of powdery mildews (Kiss 2008). It is therefore frequently used for biological control of this disease (Kiss 2003, 2008; Kiss et al. 2004; Sundheim 1982).

The biology and life cycle of *A. quisqualis* has been extensively studied (Kiss et al. 2004; Kiss 2008). Conidia of *A. quisqualis* are produced in pycnidia, which develop intracellularly in the parasitized mycelia of the powdery mildew host. In the presence of water, conidia become released and form hyphae that then penetrate the nearby hyphae of powdery mildew. *A. quisqualis* can withstand cold periods in the form of pycnidia that are saprotrophically produced in the killed plant tissues, but the fungus is not an efficient saprotroph. *A. quisqualis* is able to infect and form pycnidia within powdery mildew hyphae, conidiophores, and chasmothecia that causes reduced growth and death of the parasitic mildew (Kiss 2008).

Although ecological aspects of the mycoparasitic activity in *A. quisqualis* have been widely investigated (Angeli et al. 2011, 2012; Hashioka and Nakai 1980; Kiss 2008; Kiss et al. 2004), its molecular physiology remained largely unstudied. It is known that conidia of *A. quisqualis* poorly germinate in water or in the presence of glucose but their germination is stimulated by the presence of a water-soluble substance from the host whose chemical structure is yet unknown (Gu and Ko 1997; Sundheim 1982). Penetration of the host hyphae is made through either mechanical (Sundheim and Krekling 1982) or enzymatic processes. Rotem et al. (1999) reported the isolation of an exo-β-1,3-glucanase from *A. quisqualis*, and in vitro production of lytic enzymes has been reported for different isolates of *A. quisqualis* (Angeli et al. 2012). Siozios et al. (2015), using a high-throughput sequencing approach, established a catalog of transcripts that are formed by *A. quisqualis* during mycoparasitic interactions with *Podosphaera xanthii* (Erysiphales, Ascomycota). This catalog was then used to manufacture oligonucleotide microarrays for large-scale genome-wide analysis of transcriptional changes that occur during the early germination phase of *A. quisqualis*. They retrieved 1536 putative genes showing significant changes in transcription during the germination of *A. quisqualis*, documenting an extensive transcriptional reprogramming of *A. quisqualis* induced by the presence of the host. Genes encoding secreted proteases, virulence factors, and enzymes related to toxin biosynthesis were fund to be upregulated and interpreted as putative mycoparasitism related. They also found that a rapid activation of the transcription and translation machinery in the early stages of conidial germination is crucial for the successful transition from a dormant state to vegetative growth of *A. quisqualis*. The later phase of hyphal germination is hallmarked by upregulation of the genes involved in proteasomal and vacuolar protein degradation, protein secretion, transport, and localization, and genes related to the Snf7 family of proteins, which is involved in protein sorting and transport to lysosomal compartments (Peck et al. 2004). An involvement of these proteolytic genes in mycoparasitism has also been suggested for other fungi (Grinyer et al. 2005; Monod et al. 2002; Muthumeenakshi et al. 2007; Olmedo-Monfil et al. 2002; Zhang et al. 2015). Furthermore, the authors detected homologues of secreted proteases such as dipeptidyl-peptidase 5 and the tripeptidyl-peptidase SED3 and two putative genes with homology to the M6 family of metalloprotease domain-containing proteins which all may facilitate the penetration of the host mycelium. They also identified a small secreted protein related to the ceratoplatanin family (Chen et al. 2013; Gaderer et al. 2014; Skinner et al. 2001). They are widespread among fungi and believed to be involved in fungus–host interaction phytotoxicity in different plant pathogens (Jeong et al. 2007; Pazzagli et al. 1999) or elicitors of the plant defense response in mycoparasitic *Trichoderma* spp. (Djonović et al. 2006a; Seidl et al. 2006). The actual role of this protein in the mycoparasitic action of *A. quisqualis* remains therefore to be determined.

Siozios et al. (2015) identified genes encoding proteins involved in toxin biosynthesis among the upregulated genes: a homologue of a trichodiene oxygenase, which has a key role in

the trichothecene biosynthesis pathway (Cardoza et al. 2011), and a homologue of the sterigmatocystin biosynthesis P450 monooxygenase. Finally, two of the upregulated genes encoded multidrug transporters and the major facilitator superfamily to that resembled in *C. rosea* (Karlsson et al. 2015).

Several genes reported for their role in mycoparasitism have been found in dormant conidia of *A. quisqualis*. These were cell wall-degrading enzymes, including different glycosyl hydrolases and homologues of MAPK 1 such as *Pmk1* of *Magnaporthe grisea* (Magnaporthales, Ascomycota) and the *Tmk1* of *T. atroviride*. In fungi, MAPK signaling pathways are involved in the transduction of a wide variety of extracellular signals and play an important role in the regulation of different developmental processes, including those related to pathogenicity (Table 12.1). The authors also noted two lectin-related proteins that are well known for carbohydrate-binding properties and are widely distributed in animals, plants, and microorganisms (Lam and Ng 2010). *A. quisqualis*-related lectins could potentially be involved in the mycoparasitic process by recognizing the powdery mildew host and facilitating penetration. This study revealed several convergent strategies deployed by mycoparasites from different taxonomic groups. Future studies, including the sequencing of the *A. quisqualis* genome, could aid our understanding of the biology and evolution of the mycoparasitic lifestyle in general.

VI. Genomic Properties of *Pseudozyma flocculosa*, a Mycotrophic Basidiomycete That Evolved from an Advanced Plant Pathogenic Ancestor

Another hyperparasitic fungus that may be used to control powdery mildews is *Pseudozyma flocculosa* (Ustilaginales, Ustilaginomycotina, Basidiomycota) that is closely related to the model plant pathogen *Ustilago maydis* yet not capable to attack plants (Kemen and Jones 2012). Lefebvre et al. (2013) presented the comparative genomics of *P. flocculosa* and plant pathogenic smut fungi *U. maydis* (Kaemper et al. 2006), *U. hordei* (Laurie et al. 2012), and *Sporisorium reilianum* (Schirawski et al. 2010) (all from Ustilaginales). Several Ustilaginomycetes smut fungi share common features that are essential for pathogenicity. *U. maydis* interaction with maize (*Zea mays*) became the model system in phytopathology for investigation of factors essential for the establishment of the biotrophic parasitism. The genome sequence of *U. maydis* has revealed previously unknown genes that play key roles during such pathogenicity (Kaemper et al. 2006). Among these was a distinctive set of genes that coded for small secreted proteins referred to as effector proteins (or effectors), of which many had unknown functions. However, some were essential for infection and several counteracted plant defense responses, thus facilitating infection by the smut fungus (Brefort et al. 2009; Doehlemann et al. 2011).

In the case of *U. maydis*, the secreted effectors were found to be arranged in clusters and were upregulated upon recognition of the host plant, upon invasion, and in developing tumor tissue. Cluster deletion analysis proved their importance in pathogenicity (Kämper et al. 2006; Schirawski et al. 2010). The *P. flocculosa* genome comprises 6877 predicted protein coding genes and exhibited genomic features, including hallmarks of plant pathogenicity, that were very similar to the plant pathogens *U. maydis*, *Sporisorium reilianum*, and *Ustilago hordei* (Lefebvre et al. 2013). These findings and phylogenomic analysis suggested that *P. flocculosa* diverged from a plant pathogenic ancestor. Interestingly, however, Lefebvre et al. (2013) observed a loss of a specific subset of the secreted effector proteins (CSEP) reported to influence virulence in *U. maydis*. Although 345 CSEP-encoding genes were encoded by the *P. flocculosa* genome, which is a similar number as those found in the plant pathogenic Ustilaginales, orthologs for 51 out of 55 genes encoding secreted proteins that influence plant pathogenicity and virulence were absent in *P. flocculosa*. Since otherwise *P. flocculosa* has a high level of conservation of all other pathogenicity-related genes, e.g., encoding for enzymes in cell wall degradation and biosynthesis of secondary metabolites, this suggests that the loss of above described effectors represents the crucial factor which explains the not plant pathogenic lifestyle of *P. flocculosa*.

Yet the interaction between *P. flocculosa* and its fungal host might be dictated by other effector proteins. For example, the secretome of *P. flocculosa* includes two NPP1-containing proteins that are absent from plant pathogenic Ustilaginales (Kämper et al. 2006; Schirawski et al. 2010; Laurie et al. 2012) and also from other basidiomycetes and which are involved in the formation of necrosis and ethylene. They have so far only been identified in *Moniliophthora perniciosa* (Agaricales), the causal agent of witches' broom disease of *Theobroma cacao* (Meinhardt et al. 2008). Interestingly, the NPP1-containing proteins exhibit structural similarities to actinoporins, which form transmembrane pores (Ottmann et al. 2009), which fits well to previous observations that the collapse of powdery mildew colonies caused by *P. flocculosa* could be due to alteration of the plasma membrane and cytoplasmic leaking (Hajlaoui and Belanger 1991; Hajlaou et al. 1994; Mimee et al. 2009). Thus, NPP1-containing proteins could be key elements explaining the antagonism of *P. flocculosa* toward powdery mildews.

Other species-specific genes also provided further insights into how *P. flocculosa* acquired its potential to antagonize powdery mildews. For instance, two divergent GDSL lipases/esterases (Akoh et al. 2004) that contain a CE16 carbohydrate esterase motif that is exclusive to *P. flocculosa* have been identified that may be of relevance to its activity as an epiphytic competitor.

Another interesting observation differentiating *P. flocculosa* from the plant pathogens was the identification of a gene encoding a subgroup C GH18 chitinase adjacent to another gene encoding a chitin-binding LysM protein. The same genomic arrangement has also been found in mycoparasitic *Trichoderma* species (Kubicek et al. 2011). Interestingly, the LysM protein TAL6 of *T. atroviride* inhibited its own spore germination, while it had no effect on *Aspergillus niger* or *Neurospora crassa* (Ascomycota, Sordariales) (Seidl-Seiboth et al. 2013), suggesting a self-regulatory role in fungal growth and development. TAL6 could also act to protect the fungus against self-degradation by its other chitinases during mycoparasitism. Such a protective function for LysM chitinases against wheat (*Triticum aestivum*) was described during infection by for *Mycosphaerella graminicola* (Capnodiales, Ascomycota) (Marshall et al. 2011). While there is no evidence for a role of chitinases in the biocontrol activity of *P. flocculosa* (Bélanger et al. 2012), these finding suggests that a feature is shared with the mycoparasites, which requires further investigation.

VII. Conclusive Remarks on the Use of Mycotrophic Fungi in Agriculture

The biological control of plant diseases, or biocontrol, is an agricultural technique that is based on the use of natural hyperparasites and/or antagonists of plant pathogenic organisms to prevent or combat disease; in a broad sense, biocontrol may also include the application of plant stimulating (micro)organisms that help crops sustain abiotic stresses such as drought or salinity. It is very important to note that not all organisms but only humans[1] are capable to do biocontrol. The success of biocontrol is best defined by its result—reduced disease index for crops, but not by the mechanism of action and the type of interactions involved. Thus, efficient bioeffectors (organisms used in biocontrol) may (1) stimulate plants to induce their resistance, (2) compete with plant pathogens, (3) antagonize plant pathogens by means of secondary metabolite production, or (4) directly attack such pathogens as parasites or predators. Figure 12.5 gives an overview of biocontrol relevant interfungal interactions. Nonnutritive antagonistic interactions are depicted in pane a, while b–d demonstrate cases of parasitism among which b and c are beneficial for the plant as the "good" fungus or bioeffector attacks either plant pathogenic nematodes (b) or plant pathogenic and therefore "bad" fungi. The nature of the interaction showed in e is disputable and may be considered as either nonnutritive mutualism (plant gets stimulated while mycoparasitic fungus may find a greater diversity of host organisms) or commensalism when only plant benefits.

[1] The cases of natural agriculture as that of leaf-cutting ants are briefly discussed above.

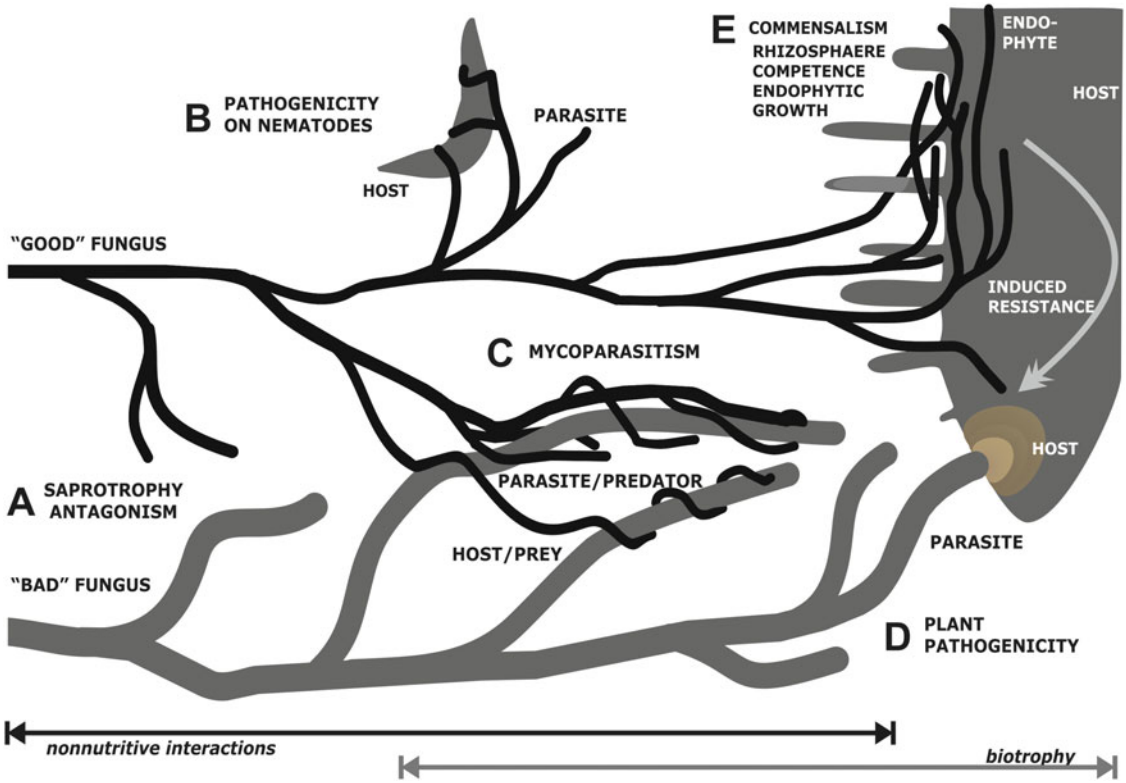

Fig. 12.5 A simplified overview of interfungal interactions that are relevant for biological control of plant pathogenic fungi and nematodes. Exclusively nonnutritive antagonistic interactions are depicted on pane (**a**), while (**b**–**d**) demonstrate cases of parasitism among which (**b**) and (**c**) are beneficial for the plant as the "good" fungus or bioeffector attacks either plant pathogenic nematodes (**b**) or plant pathogenic and therefore "bad" fungi (**c**). However, the cases of nutritive mycoparasitism (biotrophic and necrotrophic) may also be accompanied by nonnutritive interactions such as antagonism or agonism. Due to the potential applications of mycoparasitic fungi for crop protection, the so-called "bad" fungi are frequently labeled as pathogens even in the absence of their host plants when solely fungal–fungal interactions are investigated. However, in such studies, these "pathogens" serve as hosts for "good" fungi that parasitize on them; therefore, the latter ones—the "good" fungi—should rather be named as pathogens

A "good" label for a bioeffector organism is conditional and may only be applied in respect of exact interactions and an exact crop plant (Fig. 12.6). The application of mycoparasitic and antagonistic fungi for biocontrol allows to reduce the use of chemical pesticides which is usually strongly supported by the general public, and therefore respective research will likely attract more attention and funding. The Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 "establishing a framework for Community action to achieve the sustainable use of pesticides" (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009L0128) contains the respective statement: "Appropriate risk management measures shall be taken and the use of low-risk plant protection products as defined in Regulation (EC) No 1107/2009 and biological control measures shall be considered in the first place" that illustrates the future trend toward reduced use of chemical pesticides under the need to increase crop production for the growing population. However, despite the generally accepted low risk, the release of bioeffectors in the environment may also have adverse effects on both agricultural and natural ecosystems. It appears to be conceivable that introduced biocontrol fungi in case of either importation or augmentation practices will increase competition pressure for naturally present plant-beneficial microorganisms including other fungi and bacteria. For instance, the most prominent and widely accepted as "good" fungus *Trichoderma* may parasitize on arbuscular mycorrhizal fungi *Gigaspora* (Diversisporales, Glomeromycota) that are used to enhance plant nutrition and stress resistance (Lace et al. 2015) or even affect the plant as demonstrated by the colonization of

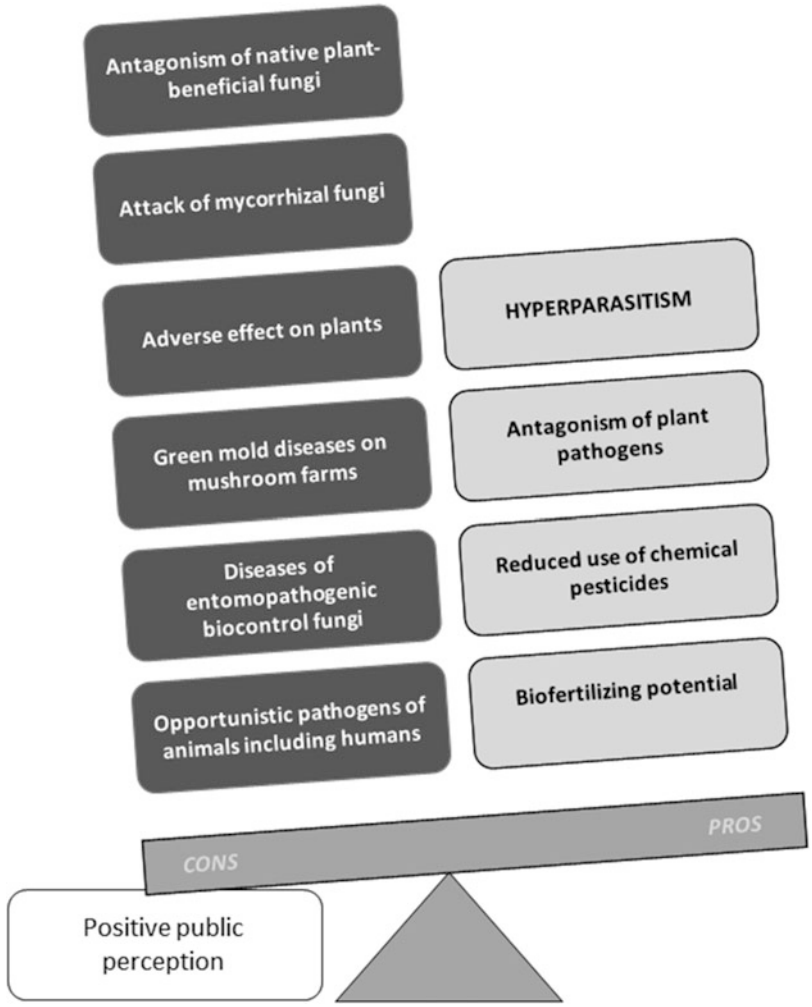

Fig. 12.6 Positive and negative arguments for the use of mycoparasitic fungi in biological control of plant pathogenic fungi

broad areas of the root epidermis of *Medicago truncatula* (Fabaceae, Angiosperms, Plantae) by *T. atroviride* leading to localized death. However, reports on direct adverse effects of biocontrol fungi on plants are rare: *T. viride* was diagnosed as a causative agent of dieback of *Pinus nigra* (Pinales, Plantae) seedling in Italy (Li Destri Nicosia et al. 2015) and different species of *Tilletiopsis* (Entylomatales, Basidiomycota) that are well-known antagonists of powdery mildews caused by Erysiphales fungi (Hijwegen 1986, 1989; Hoch and Provvidenti 1979; Klecan 1990; Knudsen and Skou 1993; Urquhart 1994). Smut fungi belonging to genus *Tilletiopsis* were demonstrated to cause "white haze" on the apple surface by Boekhout et al. (2006), in particular under conditions of ultralow oxygen storage. Clearly these fungi are able to reduce the growth of other fungi that contributes to their success as apple colonizers. The extensive colonization of harvested apples by *T. minor* and *T. pallescens* may diminish the prospects for their commercial application as biocontrol agents, as registration as a biocontrol agent will become more complicated (Baric et al. 2010).

Several studies also document the adverse effect of fungal hyperparasites on fungi used to control insect pests. It has been shown that the mycoparasitic *Syspastospora parasitica* (Hypocreales, Ascomycota) attacks *Beauveria bassiana* (Hypocreales, Ascomycota) growing on a Colorado potato beetle (*Leptinotarsa decemlineata*) cadaver (Klinger et al. 2006). Our own data indicate that this action that may also be

performed by almost any *Trichoderma* species (Druzhinina, Atanasova, unpublished) and thus the application of *Trichoderma* may counteract the positive role of *B. bassiana* on the control of the disease. Similar to this, the chytrid fungus *Gaertneriomyces semiglobifer* (Spizellomycetales, Chytridiomycota) is capable to parasitism of entomophthoralean gypsy moth *Lymantria dispar* pathogen *Entomophaga maimaiga* (Entomophthorales, Entomophthoromycota) in soil (Hajek et al. 2013). The authors propose that mycoparasitism, whether by *G. semiglobifer* or other mycoparasitic fungi, might be partially responsible for declines in azygospore reservoirs, especially under wet conditions where the motile zoospores of chytrids would have better access to susceptible fungal host spores.

Besides the direct impact on plants and plant-interacting microorganisms, fungi used in biocontrol may also have adverse effects on mushroom production (Castle et al. 1998; Hajek et al. 2013; Hermosa et al. 1999; Kim et al. 2012; Komon-Zelazowska et al. 2007; Kredics et al. 2010; Park et al. 2006) and animals including humans as opportunistic pathogens (Komon-Zelazowska 2014). Interestingly *T. longibrachiatum* that is the most frequently detected *Trichoderma* species capable to attack even immunocompetent humans (Kredics et al. 2003; Molnár-Gábor et al. 2013; Park et al. 2006; Sandoval-Denis et al. 2014) is still referred as a "good" biocontrol fungus (Ruocco et al. 2015). Moreover, the recent broad survey of clinically relevant *Trichoderma* species that was based on the detailed DNA barcoding demonstrated that almost all most prominent plant-beneficial *Trichoderma* species such as *T. harzianum*, *T. asperellum*, *T. atroviride*, *T. gamsii*, *T. koningiopsis*, and others are capable to attack immunocompromised humans (Sandoval-Denis et al. 2014). Last but not least, the materials presented in other chapters of this book on multiple and complex interactions between fungi and bacteria allow to assume the severe impact of introduced "good" but environmentally aggressive fungi on these communities, which may cause both positive and negative consequences for soil microbiome in general and consequently on plants.

Interestingly, to the best of our knowledge, up to now there are no reports published on adverse effects of *Clonostachys rosea* on humans, cultivated mushroom, or biocontrol insects. It could be possible that the mycoparasitic ability derived from herbivorous ancestors may possess fewer number of possible adverse effects compared to mycoparasites that evolved from an entomopathogenic-like organisms. No detailed ecological risk assessment analyses on the use of mycotrophic fungi have been performed. However, the newest genome-wide mechanistic and evolutionary studies would provide sufficient background for such research.

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