Chapter 6
Continuous Spectrum of Lifestyles of Plant-Associated Fungi Under Fluctuating Environments: What Genetic Components Determine the Lifestyle Transition?

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Abstract Plants interact with diverse fungal species, ranging from pathogens to beneficial endophytes. The pathogenic and beneficial lifestyles of fungi have often been studied separately and independently, so the aspects of genetic basis that contribute to lifestyle transitions in plant-associated fungi have not been generally addressed. The *Colletotrichum* genus comprises a highly diverse group of pathogens that infect and cause anthracnose diseases in a wide range of plant hosts. On the other hand, some of the *Colletotrichum* species act as beneficial endophytes and promote plant growth under conditions of stress. The presence of diverse *Colletotrichum* species with contrasting infection strategies thus provides a suitable model system in which to explore the molecular basis for discriminating pathogenic and beneficial lifestyles of plant-associated fungi. This chapter reviews recent molecular-based research related to pathogenic and beneficial *Colletotrichum* species and discusses the possible molecular basis underlying the lifestyle determination, based on the results of comparative genomics and *in planta* transcriptome analysis.

6.1 Introduction

Plants associate intimately with diverse microbes, ranging from pathogens causing disease to beneficial microbes promoting plant growth. Unlike animal guts, in which bacterial species are dominant, plants also host diverse eukaryotic fungal species. However, despite their richness and diversity in plant ecosystems, much less is known about the eco-physiological functions of fungal species than is understood for bacterial ones (Rodriguez et al. 2009). Nevertheless, several host and fungal genetic factors

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required for pathogenic lifestyles of plant-associated fungi have been identified in several plant–pathogen interaction model systems (Boller and He 2009). Similarly, genetic factors underlying the lifestyles of beneficial fungi have been identified in the context of plant interactions with mutualistic arbuscular mycorrhizal fungi that promote plant growth under nutrient-limiting conditions or for some of the root-associated endophytes such as beneficial *Serendipita indica* (Bonfante and Genre 2010; Varma et al. 1999). However, as most of the molecular-level reports related to plant–microbe interactions have focused on specific details involved in each type of association, there has been little generalization about molecular mechanisms that are critical for a selection of lifestyle as either pathogens or mutualists.

Although fungal pathogenic and beneficial lifestyles appear to be quite different, it has been reported that closely related fungal species often behave with opposite lifestyles in the same host (Hacquard et al. 2016; de Lamo and Takken 2020), suggesting that subtle genetic differences determine the lifestyles of plant-associated fungi. Furthermore, some host factors have contributed to colonization by both pathogenic and beneficial fungi (Wang et al. 2012), suggesting the presence of a common pathway for plant-associated fungi. This is also consistent with the ecological view that lifestyles of plant-associated microbes sometimes show continuity from pathogens to mutualists, depending on the host and environmental conditions (Hardoim et al. 2015). Understanding the basis for how lifestyles of microbes are determined in hosts will break down of the dogma of pathogen or mutualistic lifestyles labels, into a more continuous spectrum of lifestyle interactions.

The ascomycete genus *Colletotrichum* causes anthracnose diseases in a wide range of economically important crops, and is considered to be one of the top 10 most devastating fungal pathogens of scientific and economic importance (Dean et al. 2012). Interestingly, some of the *Colletotrichum* species are reported as saprotrophs and also as endophytes that colonize plant tissues without causing disease symptoms. The whole genome information and/or the in-depth *in planta* transcriptome data for pathogenic and endophytic *Colletotrichum* fungi have been reported (O'Connell et al. 2012; Gan et al. 2013; Hiruma et al. 2016). Thus, the accumulated information on the diverse lifestyles of *Colletotrichum* species can help to elucidate the genetic basis discriminating pathogenic and endophytic lifestyles of plant-associated fungi. This chapter first briefly summarizes the published reports on molecular examination of pathogenic and endophytic *Colletotrichum* species. Based on available comparative genome and *in planta* transcriptome analysis, the chapter then considers a possible genetic basis that can discriminate pathogenic and endophytic lifestyles of *Colletotrichum*, as well as future perspectives for identification of a genetic basis for these tendencies.

6.2 *Colletotrichum* Fungal Species as Pathogens

The large ascomycete genus *Colletotrichum* causes anthracnose diseases in a wide range of economically important crops, and has been named among the top 10 devastating fungal pathogens of scientific and economic importance (Dean et al. 2012). Many *Colletotrichum* species, as well as the rice blast fungus *Magnaporthe oryzae,* undertake a hemibiotrophic infection strategy after their invasion into the host tissues, in which an initial biotrophic phase dependent on living host cells is followed by a destructive necrotrophic phase (Perfect et al. 1999). In contrast to genuine obligate biotrophs such as powdery mildew and arbuscular mycorrhizal fungi, most of the already-described *Colletotrichum* species are readily amenable to axenic culture and genetic manipulation, which enables access to functional fungal genetic analysis. Added to this, high-quality genome sequences are available for more than 10 *Colletotrichum* species, which facilitates comparative genomics and molecular genetic studies in this fungal genus (Table 6.1, O'Connell et al. 2012; Gan et al. 2013, 2016; Hacquard et al. 2016).

Soon after a spore attaches to the surface of its host, the spore starts its morphological developmental process, along with secretion of various enzymes for host invasion (Tucker and Talbot 2001). For fungal entry trial into host leaves, the majority of reported *Colletotrichum* and other hemibiotrophic fungal pathogens such as the rice blast fungus *Magnaporthe oryzae* species form dome-shaped infection structures called appressoria soon after a spore attaches to the surface of its host (Ryder and Talbot 2015). The infection structures are heavily black-melanized, and are considered to enable the pathogenic fungus to generate enough turgor pressure (up to 6–8 MPa) to invade host tissues protected by tight cell-wall components (Kubo and Furusawa 1991; Howard and Valent 1996; Dean 1997; de Jong et al. 1997). Indeed, genetic manipulation or chemicals targeted at the melanin biosynthetic pathway are effective in inhibiting such entry and pathogenesis (Hiruma et al. 2010). Plant infection by pathogens involves secretion of effector proteins that suppress plant immunity responses and facilitate pathogen growth within plant tissues (O'Connell et al. 2012; Lo Presti et al. 2015). Interestingly, development of appressoria on host tissues is also tightly linked with secretion of effectors. It has been reported that virulence-related effectors of *Colletotrichum higginsianum* and *C. orbiculare* are focally accumulated at appressorial penetration pores (Kleemann et al. 2012), suggesting that the effectors are secreted from the pores. Infection-stage specific transcriptome analysis during leaf colonization by pathogenic *C. higginsianum* also revealed that genes encoding cell-wall-degrading enzymes are also upregulated (O'Connell et al. 2012), suggesting that pathogenic *Colletotrichum* penetrates the thick plant cell wall through the use of enzymes that degrade the host plant cell walls.

After penetration, the intracellular hyphae of most of the characterized *Colletotrichum* fungi are enclosed by the host membrane and establish a transient biotrophic phase with the host plant (Fig. 6.1). Analysis of the transcriptome during leaf colonization by pathogenic *C. higginsianum* revealed that several genes related

Table 6.1 Lists of representative available Colletotrichum whole genome information

Species	Strains	Life-styles	Hosts	References	Clade
C. fioriniae	PJ7	Pathogen	Varioius plants	Baroncelli et al. (2014a, b)	Acutatum
C. orchidophilum	IMI 309357	Pathogen	Orchid	Baroncelli et al. (2018)	Acutatum
C. salicis	CBS 607.94	Pathogen	Varioius plants	Baroncelli et al. (2016)	Acutatum
C. simmondsii	CBS122122	Pathogen	Varioius plants	Baroncelli et al. (2016)	Acutatum
C. acutatum	KC05	Pathogen	Peper	Han et al. (2016)	Acutatum
C. graminicola	M1.001	Pathogen	Maize	O'Connell et al. (2012)	Graminicola
C. sublineola	TX430BB	Pathogen	Sorghum	Baroncelli et al. (2014)	Graminicola
C. incanum	MAFF 238704, MAFF 238706, MAFF238712, MAFF238713	Pathogen	Radish, *A. thaliana,* lily	Gan et al. (2017), Hacquard et al. (2016)	Spaethianum
C. tofieldiae	0861, CBS168.49, CBS130851, CBS 495.85	Endophyte	*A. thaliana*	Hacquard et al. (2016)	Spaethianum
C. higginsianum	IMI 349063, MAFF 305635	Pathogen	*A. thaliana*	O'Connell et al. (2012), Dallery et al. (2017), Tsushima et al. (2019)	Destructivum
C. tanaceti	BRIP57314	Pathogen	Pyrethrum	Lelwala et al. 2019	Destructivum
C. shisoi		Pathogen	*Perilla frutescens*	Gan et al. 2019	Destructivum
C. chlorophyti	NTL11	Pathogen	Legumes, tomato, soybean	Gan et al. (2017)	
C. fructicola	Nara-gc5	Pathogen	Strawerry	Gan et al. (2013)	Gloeosporioides
C. fructicola	CGMCC3.17371	Pathogen	Strawerry	Armitage et al. (2020)	Gloeosporioides
C. fructicola	1104–7	Pathogen	Apple	Liang et al. (2018)	Gloeosporioides
C. gloeosporioides	Cg-14	Pathogen	Vairous Fruits	Alkan et al. 2013	Gloeosporioides
C. truncatum	MTCC no. 3414	Pathogen	chilli	Rao and Nandineni (2017)	Truncatum
C. orbiculare	104-T	Pathogen	Cucumber	Gan et al. (2013)	Orbiculare
C. lindemuthianum		Pathogen	Bean	de Queiroz et al. (2017)	Orbiculare

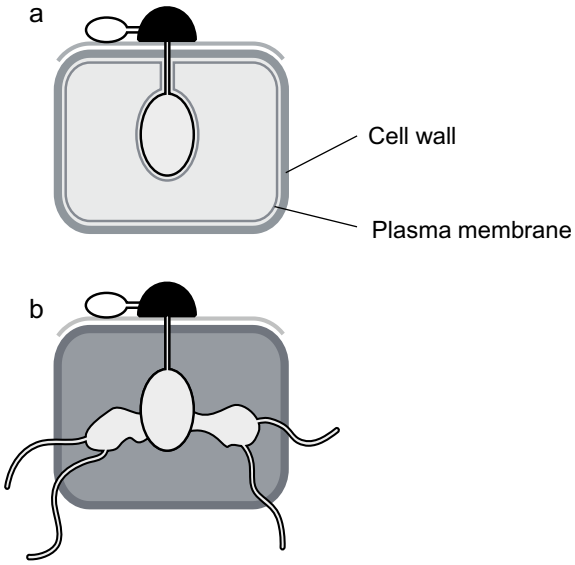

Fig. 6.1 Leaf infection process by pathogenic *Colletotrichum species*. **a** Majority of pathogenic *Colletotrichum* species form dome-shared black-melanized appressoria on leaf surface soon after the spores land in the surface. Via turgor pressure and cell-wall-degrading enzymes, the pathogens penetrate host cells and form biotrophic hyphae that are enclosed by host plasma membrane in epidermal cells. Yellow color represents cuticle layer. **b** After transient biotrophic phase, pathogenic *Colletotrichum* species turns to a necrotrophic phase during which the pathogens develop the thiner hyphae (than biotrophic hyphae) and actively kill host cells. The transition timing from biotrophic phase to necrotrophic phase is diversified among *Colletotrichum*

to effectors, which are different from genes induced during penetration, are specifically induced during biotrophic interactions. Maximum numbers of the effector candidate genes are highly induced during the biotrophic phase, so the biotrophic interface (between the fungal hyphae and plant membrane component) appears to be a site for such effector secretion. In support of this idea, virulence-related effectors of pathogenic *C. orbiculare* fused with fluorescence protein accumulated in a ring-like region around the neck of the primary biotrophic hyphae in a manner dependent on an exocytosis-related component, namely, Rab GTPase SEC4 (Irieda et al. 2014). Combining this observation with the fact that disruption of SEC4 attenuates the virulence of *C. orbiculare*, it appears that virulence effectors are secreted via ring-like regions at the interface. Some virulence-related effectors of hemibiotrophic *Magnaporthe oryzae* focally accumulate in the biotrophic interfacial complex formed in a space between the plant membrane and biotrophic hyphae, which are different from the ring-like resigns formed in biotrophic hyphae of *C. orbiculare* (Giraldo et al. 2013). These findings suggest that the mechanisms of effector delivery via fungal biotrophic hyphae could be diverse among hemibiotrophic pathogens. This contrasts with the case of well-conserved pathogenic bacterial strategies to inject effectors into the cytosol of eukaryotic cells via a type III secretion system (Hueck 1998).

It would be interesting to investigate whether formation of ring-like structures is specific to *C. orbiculare* or is widespread across the *Colletotrichum* genus. In addition to effectors, it is noteworthy that several genes related to secondary metabolism are also highly up-regulated during the biotrophy (O'Connell et al. 2012, See also the section of "Repertories of secondary metabolites in pathogenic versus beneficial *Colletotrichum*").

The biotrophic phase is transient and suddenly switches to a neurotrophic phase. During the neurotrophic phase, pathogenic *Colletotrichum* fungi differentiate thin, rapidly growing hyphae that kill host tissues (Fig. 6.1). During the necrotrophic phase, *Colletotrichum* pathogens induce genes encoding cell-wall-degrading enzymes that target different types of plant cell-wall components: proteases, necrosis-inducing proteins, and secondary metabolites including several putative fungal toxins (O'Connell et al. 2012). This transcriptomic reprogramming during necrotrophy appears to be adapted to kill host cells and to retrieve nutrients from host tissues. However, the mechanisms by which fungi start transition from late biotrophy to necrotrophy are not clear.

6.3 *Colletotrichum* Species as Endophytes

In addition to hemibiotrophic pathogenic species, the *Colletotrichum* genus has endophytic *Colletotrichum* species that colonize inside plant tissues without causing disease symptoms and that in some cases confer benefits to the host plants. Many reports show that various *Colletotrishum* species have been isolated from various healthy plants after surface disinfection, suggesting these strains are endophytes living inside host tissues without causing visible disease, at least as applies to the moment when they were isolated. However, infection processes of most of the isolated putative endophytic *Colletotrichum* species have not been further characterized in the laboratory or in the field, so it is still not clear whether the *Colletotrichum* fungi isolated from healthy plants after surface disinfection associate with host plants as true endophytes or just as stochastic encounters or as pathogens for which the virulence is suppressed via mechanisms as yet unknown. Importantly, however, it has been reported that *C. tofieldiae* isolated from and frequently detected in several different healthy wild *Arabidopsis thaliana* populations in Spain asymptomatically colonizes the host roots and, importantly, promotes plant growth under low-phosphate conditions, in part by transferring phosphorus to the host via the hyphae (Fig. 6.2, Hiruma et al. 2016). This resembles the action of arbuscular mycorrhizal fungi that promote plant growth via nutrient transfer (Bonfante and Genre 2010). In contrast to arbuscular mycorrhizal fungi that receive carbon from host plants, however, what kinds of benefits *C. tofieldiae* receive when the fungus transfers phosphorus to the host plants is still an open question.

Interestingly, in-depth microscopic analysis revealed that although *C. tofieldiae* causes epidermal cell death after the transient biotrophic phase, *C. tofieldiae* appears to form a stable biotrophic interface with host plants in cortex cells, the second

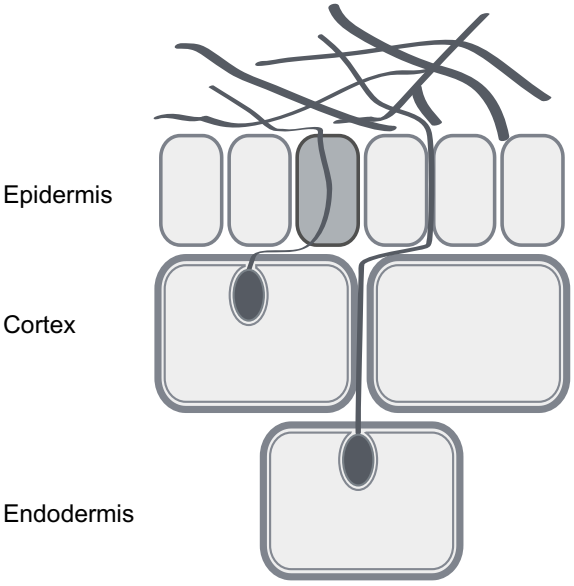

Fig. 6.2 Root infection process by the beneficial endophytic *Colletotrichum tofieldiae (Ct)*. Hyphae of *Ct* start to penetrate epidermal root cells without forming appressoria. After transient biotrophic interactions in epidermal cells, the infected epidermal cells lost viability. Hyphae of *Ct* also localizes in intercellular regions. In cortex or endodermis, hyphae of *Ct* are enclosed by host plasma membrane and appears to form stable biotrophic interactions. The transition from biotrophy to necrotrophy has not been observed in *A. thaliana* Col-0 plants

layers of plant root cells. The morphological features of the interface are similar to those of the transient biotrophic interface formed by the majority of pathogenic *Colletotrichum*. However, transition from biotrophy to necrotrophy, as observed in most of the pathogenic *Colletotrichum*, is not observed during *C. tofieldiae* root colonization, and the absence of this transition might thus prevent disease symptoms during fungal colonization. At the genome level, however, *C. tofieldiae* is very closely related to root-infecting pathogenic species belonging to the spathianum clade, such as *C. incanum* (Hacquard et al. 2016). Indeed, even *C. tofieldiae* displays high virulence in host *cyp79B2 cyp79B3* mutant plants lacking host tryptophan-derived antimicrobial metabolites, including the phytoalexin camalexin and indole glucosinolates (Hiruma 2019; Hiruma et al. 2016). This in turn suggests that the antifungal metabolite pathway is required to suppress the potential pathogenesis of *C. tofieldiae*.

There are diverse ranges of infection strategies that range from pathogenic to beneficial in *Colletotrichum* species. What is the molecular basis that discriminates pathogenic from beneficial infection strategies? The infection strategies for pathogenic and beneficial action appear to be quite different. However, it has been reported that deletion of one genetic locus turns pathogenic *C. magna* to an endophyte that protects the host plants from pathogens (Freeman and Rodriguez 1993; Redman

et al. 1999). Similarly, deletion of one plant tryptophan-derived metabolite pathway is enough to turn beneficial *C. tofieldiae* or another beneficial endophyte *Serendipita indica* to a pathogen, under the condition where the beneficial fungi promotes the growth of some wild-type plants (Nongbri et al. 2012; Lahrmann et al. 2015; Hiruma et al. 2016). These results suggest that lifestyles of plant-associated *Colletotrichum* could be determined using a tractable genetic basis. Such high phylogenetic relatedness to pathogenic species seems to be common, rather than exceptional, for endophytic fungi isolated from healthy, surface-sterilized tissues of different plant species (Rodriguez et al. 2009). Thus, as a first step to identifying the genetic basis discriminating beneficial and pathogenic lifestyles, it is very useful to perform comparative analysis that includes cytological, genomic, and *in planta* transcriptome analysis using *Colletotrichum* species with diverse lifestyles as a model (Table 6.1).

6.4 Apparent Lack of a Transition from Biotrophy to Necrotrophy in Beneficial *Colletotrichum*

As described in the preceding sections, microscopic analysis suggests that beneficial *C. tofieldiae* does not show a transition from biotrophy to necrotrophy, which contrasts with a few characterized root-infecting pathogenic *Colletotrichum* species (Sukno et al. 2008). Arbuscular mycorrhizal fungi also form a biotrophic interface called arbuscule in cortex cells and do not show transition to any necrotrophy (Bonfante and Genre 2010). Rather, the old arbuscule is degraded by host plants (Kobae et al. 2016). Although the molecular mechanisms underlying the transition from biography to necrotrophy are not yet understood, *C. higginsianum* mutants defective in pathogenicity as a result of *Agrobacterium*-mediated insertion of T-DNA in a genomic region do not show a transition from biotrophy to necrotrophy (Huser et al. 2009). One *C. higginsianum* mutant lacking a mini chromosome also failed to switch to necrotrophy (Plaumann et al. 2018). It is not currently clear what defects cause the *C. hissinsianum* mutants to fail to switch to necrotrophy. Interestingly, a recent study suggests that penetration hyphae of *C. orbiculare* mutants lacking the homeobox transcription factor CoHox1 did not turn to necrotrophy even after 19 days post inoculation (Yokoyama et al. 2019), suggesting that CoHox1 is involved in the transition from biotrophy to necrotrophy. It is also interesting to address whether the prolonged biotrophic phase observed in the mutants causes any effects on plant growth and development, especially under stress conditions in which beneficial fungi often provide fitness benefits to host plants. A more detailed analysis of these nonpathogenic mutants, including time-resolved RNAseq analysis, as well as discovering the causative genes responsible for the phenotype, will also help investigators to understand how beneficial fungi regulate the transition (restrict necrotrophy), despite the fact that they share very similar genomes with relatively pathogenic *Colletotrichum.*

6.5 Repertories of Cell-Wall-Degrading Enzymes in Pathogenic Versus Beneficial *Colletotrichum*

The plant cell wall, composed of a matrix of pectin, hemicellulose, lignin, and structural proteins, is a barrier that prevents pathogen infection (Kubicek et al. 2014). Hemibiotrophic pathogens induce sets of cell-wall-degrading enzymes during host infection (O'Connell et al. 2012). Comparative genomics between pathogenic *Colletotrichum* fungi show that repertories of cell-wall-degrading enzymes are different, depending on which host plants the pathogens preferentially colonize (King et al. 2011; O'Connell et al. 2012). For example, *C. higginsianum* encodes more than twice as many pectin-degrading enzymes as does *C. graminicola*, which appears to be well reflected in their host preferences for plants (dicot plants have more pectin than monocot plants). In contrast, most of the characterized beneficial fungi such as arbuscular mycorrhizal fungi and ectomycorrhizal fungi have reduced repertories of genes encoding cell-wall-degrading enzymes (Tisserant et al. 2013; Nagendran et al. 2009; Martin et al. 2008). However, comparative genomic analysis of beneficial *C. tofieldiae* versus pathogenic *C. incanum*, both of which infect roots of *A. thaliana*, reveals that beneficial *C. tofieldiae* have similar repertories of cell-wall-degrading enzymes. Restriction of repertories of cell-wall-degrading enzymes has not been described for other beneficial endophytic fungi such as *Serendipita indica*, *Harpophora oryzae*, and *Helotiales* (Zuccaro et al. 2011; Xu et al. 2014; Almario et al. 2017). Furthermore, *in planta* transcriptome analysis has revealed that beneficial *C. tofieldiae* strongly expresses fungal genes encoding cell-wall-degrading enzymes during the root colonization, especially during the late colonization phase, during which *C. tofieldiae*-mediated plant growth promotion is clearly detected. These gene-encoded cell-wall-degrading enzymes act on all major polymers, including cellulose, hemicellulose, and pectin (Hacquard et al. 2016). Considering that *C. tofieldiae* promotes seed production as well, these results suggest that the absence or presence of these genes might be not key in distinguishing pathogenic from beneficial, at least in the case of the *Colletotrichum*. Unlike the case of obligate biotrophy as in arbuscular mycorrhizal fungi, the characterized endophytes including *C. tofieldiae* can be grown in the absence of host plants, and its colonization in host plants partially induced cell death, such as in epidermal cell layers (Deshmukh et al. 2006; Hiruma et al. 2016). Thus, the contrasting differences between biotrophs and hemibiotrophs might rather determine the differences in repertories and *in planta* induction of cell-wall-degrading enzymes. The roles of cell-wall-degrading enzymes during root colonization by beneficial fungi merit future in-depth studies.

6.6 Repertories of Secondary Metabolites in Pathogenic Versus Beneficial *Colletotrichum*

Filamentous fungi produce diverse secondary metabolites. Fungal secondary metabolites are of intense interest due to their pharmaceutical (antibiotics) and/or toxic (mycotoxins) properties (Yu and Keller 2005). Especially, whole genome analysis of *Colletotrichum* has demonstrated the high potential of *Colletotrichum* species for secondary metabolite production compared with relative plant-associated fungi, a factor that can be assumed from the higher numbers of secondary metabolism clusters (O'Connell et al. 2012). More than 100 secondary metabolites have been isolated from pathogenic and endophytic *Colletotrichum* species (Kim and Shim 2019). For example, several antimicrobial compounds, and plant hormones such as indole-3-acetic acid, have been isolated from a liquid culture of several *Colletotrichum* species (Zou et al. 2000; Lu et al. 2000). In terms of the regulatory mechanisms of secondary metabolism clusters, it was recently reported that some of the secondary metabolism clusters have been silenced in the absence of host plants via H3K4 trimethylation (Dallery et al. 2019), a finding that appears similar to results in *Aspergilli* and *Saccharomyces cerevisiae* (Bok et al. 2009; Palmer et al. 2013; Shinohara et al. 2016). However, although fungal mutants can lose the ability to produce particular secondary metabolites and can lose the ability to infect, little is so far known about the functions of such secondary metabolites during infection. Interestingly, exogenous application of higginsianin B, produced from one of the secondary metabolism clusters regulated by H3K4 trimethylation in pathogenic *C. higginsianum* during the penetration to biotrophic phase, suppresses jasmonate-mediated plant defenses, likely via inhibition of 26S proteasome-dependent degradation of JAZ proteins (Dallery et al. 2020). Importantly, Markov cluster algorithm (MCL) analysis comparing *C. tofieldiae* and its pathogenic relative *C. incanum* revealed that the beneficial *C. tofieldiae* possesses many more gene families for secondary metabolite biosynthesis (Hacquard et al. 2016), implying critical roles for secondary metabolites in the lifestyles of beneficial fungi.

6.7 Repertories of Candidate Effectors, Comparing Pathogenic Versus Beneficial *Colletotrichum*

During the evolutionary arms race between host plants and pathogens, plant-associated pathogens have developed several different effectors to effectively suppress host defense responses (see Hogenhout et al. 2009; Raffaele and Kamoun 2012; Sanchez-Vallet et al. 2018 evolutionary development of pathogenic effectors from genome aspect). The mutualistic arbuscular mycorrhizal fungi and ectomycorrhizal fungi also use effectors to manipulate the host hormonal pathway to promote infection (Kloppholz et al. 2011; Plett et al. 2011). Thus, it is conceivable that plant-associated microbes may preferably increase the diversity of effector

repertories to effectively suppress host defense responses. However, the numbers of annotated candidate effectors in beneficial *C. tofieldiae* are smaller than those in pathogenic *C. incanum* (Hacquard et al. 2016). In addition, time-resolved *in planta* transcriptome analysis has suggested that activation of effector genes in *C. tofieldiae* is weaker than for those of *C. incanum*. Reduced repertories of candidate effectors in genomes of endophytes compared with those of pathogens have been reported also in *Fusarium* (de Lamo and Takken 2020). As introduced in the preceding sections, effectors have a necessary role in colonizing host tissues by suppressing plant immunity and other responses. At the same time, host plants have developed resistance genes encoding nucleotide-binding leucine-rich repeat proteins (NLRs) to directly or indirectly detect the activities of effectors for termination of pathogen growth in host tissues (Bergelson et al. 2001). Indeed, it has been reported that only two plant NLRs namely ZAR1 and CAR1 in *Arabidopsis thaliana* are predicted to be responsible for detection of the majority of bacterial type III secreted effectors (more than 90%) that are distributed in populations of pathogen *Pseudomonas syringae* (Laflamme et al. 2020). This proposes unexpected broad spectrum defense responses against pathogens via effector recognition by NLRs. In addition, some of the effectors expressed during biotrophic to necrotrophic stages cause cell death responses when they are overexpressed in fungi or in plants. For example, overexpression of a biotrophy-specific *C. truncatum* effector in *C. truncatum* or in the rice blast pathogen *Magnaporthe oryzae* causes incompatibility with the host lentil and barley plants, respectively, by inducing cell death responses in infected host cells with the biotrophic hyphae (Bhadauria et al. 2013). Overexpression of *NIS1* of *C. orbiculare,* which is expressed during the biotrophic phase and has roles in suppressing plant immunity, induces cell death responses in *Nicotiana benthamiana* (Yoshino et al. 2012; Irieda et al. 2019). Overexpression of necrosis-inducing factors of *C. higginsianum*, which is expressed during necrotrophic phase induces necrotic cell death responses in *Nicotiana benthamiana* (Kleemann et al. 2012). It is not clear whether the effector-mediated cell death responses are among the essential functions of the effectors or are results of counterdefense responses by host plants, possibly via NLRs. In any case, the fact that transitions from biotrophy to necrotrophy have not been observed during wild-type *Arabidopsis thaliana* root colonization by *C. tofieldiae* suggests that the beneficial *C. tofieldiae* reduces the repertory and the expression of effectors that directly or indirectly cause cell death responses to prevent unnecessary cell death responses in host plants. However, the *C. tofieldiae* turns into a pathogen with apparent necrotrophic growth in *Arabidopsis thaliana* plants lacking tryptophan-derived secondary metabolism, including antifungal indole glucosinolates and Camalexin (Hiruma et al. 2016), suggesting that *C. tofieldiae* retains at least minimum sets of effector repertories to cause disease symptoms in susceptible host plants. It is important to address how the beneficial *C. tofieldiae* colonizes host roots and promotes plant growth with smaller repertories of effectors, as well as weaker expression of the effector genes, than the relative pathogen *C. incanum*. It is also important to investigate the mechanisms by which the majority of candidate effectors (more than 100) in the *C. tofieldiae* genome remain silent during host infection.

6.8 Conclusion

As introduced in the preceding sections, comparative genomics and *in planta* transcriptome analysis between pathogenic and beneficial *Colletotrichum* species hint at the molecular mechanisms that discriminate pathogenic and beneficial lifestyles of fungi. To validate whether these candidates are really involved in transition of lifestyles of fungi is an important task in the future. For functional analysis, establishing a mutualistic, pathogenic, or beneficial plant–microbe association model in *A. thaliana* would promote a detailed and precise understanding of the mechanisms. In bacteria, comparative genomics between several pathogenic, commensal, and beneficial *Pseudomonas* species in *Arabidopsis thaliana* have identified that a bacterial factor shared among *Pseudomonas* species, probably via horizontal transfer, is responsible for determination of the lifestyles (Melnyk et al. 2019). However, due to the fact that very limited numbers of beneficial fungal endophytes have so far been well characterized for in-depth comparative analysis between pathogenic and beneficial fungi (e.g., Table 6.1, in *Colletotrichum*), little is known about factors determining lifestyles of fungi. Therefore, there is an urgent need to identify and characterize more endophytic fungi in order to obtain insights from comparative genomics and *in planta* transcriptome analysis. Compared to animals, plants have unique features that allow them to intimately associate with eukaryotic fungi. Understanding the still largely hidden lifestyles of plant-associated fungal species will increase the understanding of plant stress adaptation strategies and of molecular mechanisms that determine the evolutionary origin of pathogenesis and mutualism.

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