

Role of Diverse Non-Systemic Fungal Endophytes in Plant Performance and Response to Stress: Progress and Approaches

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Abstract Plant–fungal symbiotic associations are ubiquitously distributed in natural plant communities. Besides the well-studied mycorrhizal symbiosis and grass systemic clavicipitaceous endophytes, recently, nonsystemic and horizontally transmitted fungal endophytes serving as plant symbionts have been increasingly recognized. Pure culture isolation and culture-independent molecular methods indicate that all parts of healthy plant tissues potentially harbor diverse and previously unknown fungal lineages. Limited evidence also supports a hypothesis that endophytic mycobiota dynamics may have a role in evolution of plants. High variability or “balanced antagonism” can be generally characterized with host–endophyte interactions, which implies that the outcome of symbiotic interactions can fall within a continuum ranging from mutualism to commensalism, and ultimately pathogenicity. Despite this complicated system, admittedly, fungal endophytes really endow the host with an extended phenotype. Accumulating facts illustrate that plant nutrition acquisition, metabolism, and stress tolerance may be strengthened or modulated via fungal symbionts. *Piriformospora indica*, a member of the order Sebaciales, simultaneously confers host resistance to biotic and abiotic stress. The ecological relevance of other fungal groups, including foliar endophytes, root dark septate endophytes (DSEs), some opportunistic and avirulent

microsymbionts (for example, *Trichoderma* and *Fusarium*), and even uncultured fungi structurally and physiologically integrated with host tissues, are also being deeply exploited. Production of bioactive metabolites by fungi, overexpression of stress-related enzymes, and induced resistance in hosts upon fungal colonization are responsible for direct or indirect beneficial effects to hosts. More knowledge of endophyte-mediated enhancement of host performance and fitness will offer alternatively valuable strategies for plant cultivation and breeding. Meanwhile, with unprecedented loss of biodiversity, discovery of indigenously novel symbiotic endophytes from natural habitats is urgently needed. In addition, we present some approaches and suggestions for studying host–endophyte interactions.

Keywords Fungal endophytes · Symbiosis · Stress tolerance · Diversity · Metabolism

Introduction

Symbiosis, defined as “the permanent association between two or more specifically distinct organisms, at least during a part of the life cycle” (de Bary 1879), is known to be a ubiquitous and important aspect of life on Earth. Most animals and plants live in close associations with a series of microorganisms. Evolutionarily, plants require some specialized microbial partners for adapting to certain ecological niches to maintain their normal growth and development. Rhizobium, actinorhizal and mycorrhizal symbionts have long been investigated and viewed as the primary mutualistic microbial symbionts associated with plant roots (Pawlowski and Bisseling 1996; Diouf and others 2003). In addition, aboveground plant–fungal mutualistic interactions also exist in some temperate and

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tropical grasses and it is well known that endophytic systemic clavicipitaceous fungi colonize inner grass leaf tissue and exert beneficial effects on hosts through increased resistance to herbivores, pathogens, and drought stresses (Kuldau and Bacon 2008).

Most agree that endophytes are fungi that live internally and remain asymptomatic for at least part of their life cycle (Wilson 1995). Thus, endophytes encompass a wide range of fungi, including latent pathogens and dormant saprophytes. However, recent phylogenetic data demonstrate that some endophytes are genetically distinct from known parasites in the same host despite their morphological identity (Ganley and others 2004). Carroll (1988) defined two different types of endophytic fungi: constitutive mutualists (type I endophyte) and inducible mutualists (type II endophyte). It is usually proposed that most type I clavicipitaceous endophytes (*Epichloil/Neotyphodium*) are systemic and vertically transmitted through seeds and exclusively infect grass. Instead, nonsystemic type II endophytes are taxonomically diverse, horizontally transmitted from plants to plants, and colonize all plants in ecosystems (Rodriguez and others 2008).

Recently, nonsystemic endophytic fungi identified in a very wide range of host plant species have met with increasing attention due to their striking species diversity and multiple functions (Rodriguez and others 2008). Unlike other plant-microbe symbiotic relationships, however, plant–fungal endophyte associations generally occur in both aboveground and belowground plant tissues (Faeth and Fagan 2002). Strongly supported evidence has revealed that type II endophytes represent more or less phylogenetic diversity when compared to type I endophytes and mycorrhizal symbionts (Arnold and others 2002; Faeth and Fagan 2002; Vandenkoornhuyse and others 2002). It has been suggested that the endophyte and its host is a balanced antagonism or conditional mutualism (Schulz and Boyle 2005; Donoso and others 2008), which depends on the status of two partners. The plant's physiology and genotype, the genotype and virulence of the fungi, together with the environmental context ultimately determine the outcome of plant-endophyte interactions (Freeman and Rodriguez 1993; Redman and others 2001; Donoso and others 2008). It appears that variability is the nature of the endophyte-plant interaction.

However, we propose that despite the complex and labile associations, recent advances in microbial ecology have demonstrated the pivotal roles of type II endophytes in improving host growth, fitness, and stress responses (Waller and others 2005; Porrás-Alfaro and others 2008; Rodriguez and others 2008). In some cases, harboring these symbiotic fungi is indispensable to the survival of hosts, implying the evolution of life history strategies of plants (Redman and others 2002). In this review article we try to

outline the current knowledge of type II fungal endophytes with special reference to some well-studied fungal lineages, and focus on their beneficial physiological interactions with hosts, which may expand our views on how plants accommodate fungal endophytes to adapt to adverse conditions and improve nutrition acquisition in a competitive ecological community.

Diversity of Type II Fungal Endophytes and Implications for Plant Evolution

Estimation and Characterization of Endophytic Fungal Diversity with Traditional and Molecular Tools

Fungi are an important key group of diverse microorganisms in terrestrial ecosystems because of their pivotal role in the carbon cycle. They also directly establish intimate relationships with plants, animals, and other organisms. It has been estimated that there are no fewer than 1.5 million fungal species on Earth (Hawksworth 2001), whereas a minimum number of 712,000 fungal species has also been estimated (Schmit and Mueller 2007). However, an ITS-based survey of environmental samples yields soil fungal species richness ranging from 3.5 to 5.1 million (O'Brien and others 2005). There is still debate on the true scale of fungi in nature. In any case, there is no doubt that the remarkably diverse fungal species make great contributions to ecological patterns and processes.

From numerous investigations, there is now growing evidence that endophytic fungi represent formerly uncharted fungal lineages and comprise vast amounts of fungal diversity on a global scale (Arnold and others 2002; O'Brien and others 2005). Frequently, some recovered isolates cannot be identified as they are sterile or ITS sequences do not resemble any described species in the database, potentially being assigned to a new taxonomic class. Therefore, the isolation and characterization of the fungal community associated with a wide range of hosts not only helps us estimate the diversity of fungi but provides raw material for further investigation of interactive mechanisms of the two partners (Collado and others 2007). It was pointed out recently that the dilution-to-extinction culture method has been used successfully to recover more diverse endophytic fungi species from leaf tissues than the segment plating method. This high-throughput cultivation procedure provides promise for further basic endophyte biology research (Collado and others 2007; Unterseher and Schnittler 2009). In addition, ecological indices, including Fisher's α , Shannon diversity index (H), Simpson's diversity index (D), Margalef's richness index (R1), and species accumulation curves (generated by *EstimateS*, <http://viceroy.eeb.uconn.edu/EstimateS>), are used to evaluate

the fungal diversity in plants (Suryanarayanan and Kumaresan 2000; Hoffman and others 2008; Tao and others 2008).

Meanwhile, culture-independent molecular methods offer the possibility of better understanding the mycobiota that naturally reside in the inner tissues of plants (Arnold and others 2007) and are recognized as an important complement to conventional culture methods. Direct clone library sequencing, high-throughput sequencing, DGGE, and PCR-AFLP are the prevailing tools used for molecular analysis (Allen and others 2003; Nikolcheva and Bärlocher 2005a, b; O'Brien and others 2005; Peay and others 2008; Zuccaro and others 2008). It is also critical to note that *Fungal Environmental Sampling Network* (FESIN, <http://www.bio.utk.edu/fesin/>), a well-established platform, has been initiated to improve the knowledge of fungal identity, diversity, and ecology with advanced molecular and phylogenetic tools.

Increasing research focuses on the endophytic fungal community of plants exposed to extreme environments or that occupy unique niches (El-Morsy 2000; Collado and others 2002; Barrow and others 2004b; El-Zayat and others 2008; Porrás-Alfaro and others 2008; Maciá-Vicente and others 2008a). It is reasonable to speculate that such unique ecological niches may sustain diverse or novel fungal species contributing to the host's response to external stress signals. A novel endophytic *Curvularia* sp., isolated from a thermotolerant plant (*Dichantheium lanuginosum*), increases host heat tolerance (Redman and others 2002). Apart from the recognized fungal species diversity and community of woody plants, direct clone library sequencing detects extensively distinct fungal groups occurring in desert perennial grass roots (Vandenkoornhuysen and others 2002; Porrás-Alfaro and others 2008) and reveals that dark septate endophytes (DSEs) within the order of Pleosporales dominantly encounter roots and 51 operational taxonomic units (OTU) have been recorded (Porrás-Alfaro and others 2008). *Dendrobium nobile*, an epiphytic orchid, hosts at least 33 different nonmycorrhizal fungal morphospecies belonging to 14 genera (Yuan and others 2009). Surprisingly, our data also suggest a rich endophyte assemblage in the roots of wild rice (*Oryza granulata*), a gramineous plant in China. Thirty-one distinct ITS genotypes based on cultured isolates and 35 potentially unique phylotypes generated by direct PCR were determined despite their low colonization rate (unpublished data). These results provide additional evidence that endophytes residing in annual or perennial herbaceous plants may be as rich as those found in woody plants (Saikkonen and others 1998).

Admittedly, there is certainly a great body of literature describing the endophytic fungal diversity associated with a wide range of hosts. The question remains: What roles do these cohabiting colonizers play in natural ecosystems?

Do Endophytic Fungal Population Dynamics Drive Plant Evolution?

Currently, the hologenome theory of evolution hypothesizes that the diversity of microbial symbionts associated with animals and plants plays an important role in the nutrition and the adaptation of hosts to a stressful environment, thus evolving the holobiont (Rosenberg and others 2007; Zilber-Rosenberg and Rosenberg 2008). The authors emphasize the importance of diversity of microbiota in the evolution of the hosts because the genetic diversity of the microbial consortium can extend the range of the environment in which hosts will successfully survive (Zilber-Rosenberg and Rosenberg 2008). It is also recognized that the diversity and ecological roles of fungal endophytes were previously underestimated and that they contribute benefits to their hosts in multiple ways (see below). These fungi may influence the plant metabolic state, mainly through communication and transduction between the two partners, and contribute genes and relevant bioactive products that enable plants to successfully respond to biotic and abiotic stresses (Barrow and others 2008). Considering the rich endophytic assemblage, even some unculturable endophytes are structurally integrated with the host's inner tissues; it is therefore not surprising that this fungal consortium will have a major influence on plant adaptation and evolution (Barrow and others 2008). It can be proposed that the plant-endophyte functional units are better prepared to face stress situations.

Type II Fungal Endophyte–Host Relationships: A General and Novel System for Studying Mutualistic Plant–Fungal Interactions

Considerable information on plant–fungal interactions involving mycorrhizal symbiosis and pathogenic interactions is available. The recent literature has provided more evidence that some common symbiosis (SYM) signaling pathways are present in plants for harboring different beneficial microbes (Gutjahr and others 2008). Plants have also evolved ancient and conserved biochemical events and gene regulation for responding to mycorrhizal fungi and pathogenic fungi colonization (Güimil and others 2005; Kogel and others 2006). However, host-specific molecular mechanisms for guarding against endophytic fungi entry are possibly different from other fungal groups (Gutjahr and others 2008). To gain insight into the physiological interactions and signal exchanges of endophytes and their hosts, establishment of in vitro model systems for assessing host interactions with endophytic fungi is required. The molecular dialogue and gene regulation for maintaining grass-endophyte mutualistic associations are beginning to

be revealed (Tanaka and others 2006). High host specificity and low fungal species diversity are characteristic of grass-endophyte associations; this appears to be a unique and special case. Preferentially, type II fungal endophytes represent an interesting experimental model of host–endophyte interaction because of their ubiquity and diversity (Scannerini and others 2002; Van Bael and others 2005). Host cell suspensions, calli, and plantlets can be cocultured with fungal endophytes under controlled and axenic conditions for analyzing the metabolic changes in both partners (Peters and others 1998; Mucciarelli and others 2003; Baldi and others 2008; Li and Tao 2009).

The next important issue to be resolved is how to establish an appropriate model system for revealing species interactions that influence plant growth and development. Based on personal communication, experience, and the work of others, we present here some suggestions:

1. The easy and short-term procedure for mass propagation of aseptic plantlets in vitro. Availability of seeds of herbaceous and woody plants provides the prerequisite for subsequent analysis. *Theobroma cacao* L., *Arabidopsis thaliana* (L.), and important crop plants may be good alternatives (Arnold and others 2003; Peskan-Berghofer and others 2004; Rodriguez and others 2008).
2. Information is available on the genetic background of the host plant. At least some metabolic pathways or other phenotype-related genes are well understood, which is necessary to demonstrate the ecophysiology and gene expression profiles of hosts upon fungal colonization. *Mentha piperita*, *Artemisia annua*, *Camptotheca acuminata*, and other model plants are ideal candidates (Wang and others 2001; Mucciarelli and others 2003).
3. Stress-adapted and non-cultivated status of plants probably host unique, rich and novel indigenous fungi. The no host-specificity of these fungi makes them a potentially good fungal resource for applying to native and nonhost plants (Hung and others 2007; Barrow and others 2008; Thomas and others 2008).

Principles, Methods, and Techniques in Studying Plant–Fungal Endophyte Systems

A great deal of knowledge has been compiled about mycorrhizal associations and plant-pathogenic fungi interactions, and related tools and techniques are already available. Given that endophyte–plant interactions share similar evolutionary and ecological processes with other host-mutualist, host-parasite, and host-disease interactions (Saikkonen and others 2004), it seems likely that most

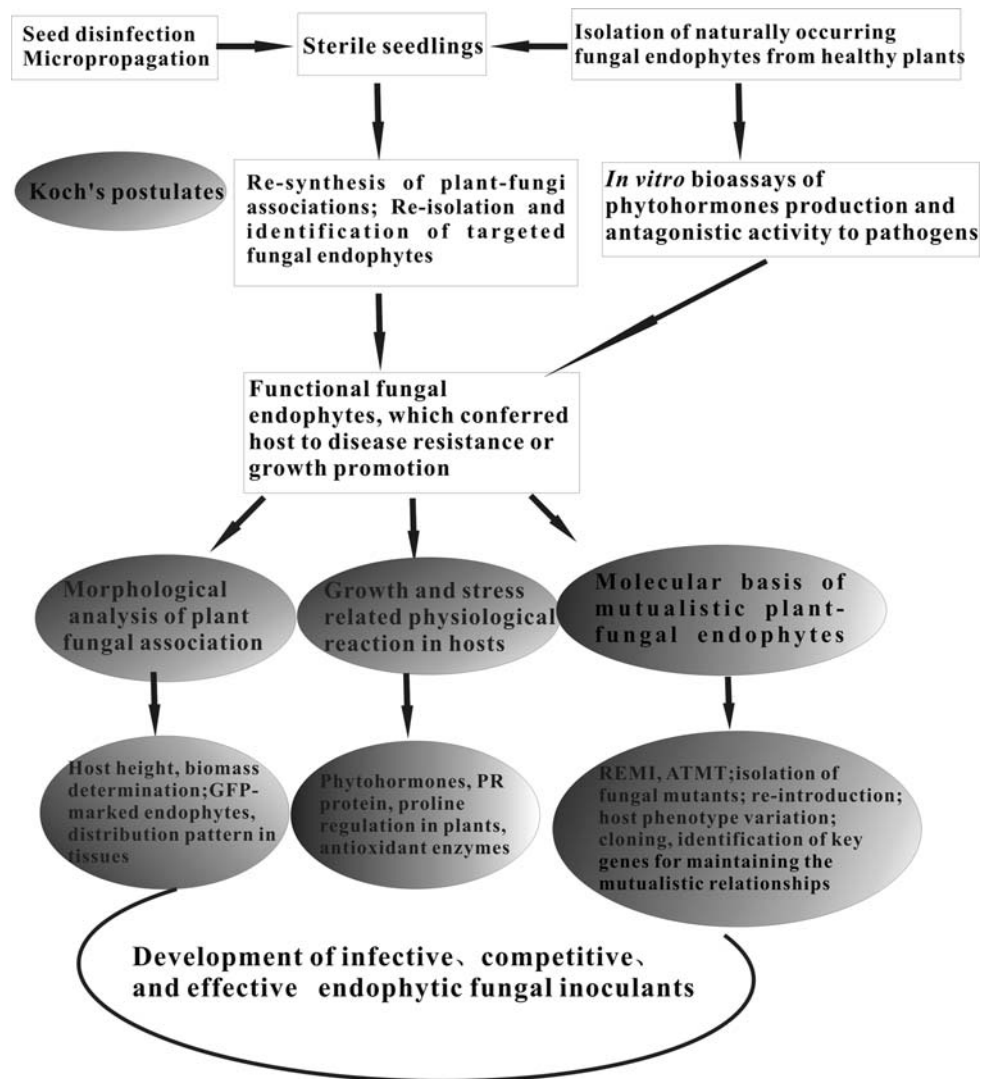
approaches and principles applied in plant pathology and mycorrhizal biology are also appropriate for studying endophyte biology. Here, we outline some generally accepted views and methods from the published literature (Arnold and others 2003; Tanaka and others 2006; Grunewaldt-Stöcker and others 2007; Kharkwal and others 2007; Shahollari and others 2007; Kempainen and others 2008) (Fig. 1).

Host Secondary Metabolite Profiles Affected by Endophytic Colonization

Plant-associated microbes, if any, can exert beneficial, neutral, or detrimental effects on plant performance. Signals released from the two partners and the process of recognition and transduction will strongly modulate the host metabolic state and level to determine whether the interaction is compatible or incompatible (Nurnberger and Lipka 2005). In rhizobium and mycorrhizal symbiotic associations, conserved signaling pathways and molecular dialogue events are of great importance to the maintenance of functional symbiosis, thus accompanied by the differential gene expressions and transcript regulation in roots, ultimately resulting in marked changes of primary and secondary root metabolism (Savouré and others 1994; Fujihara and others 2006). For example, stimulation of carotenoid metabolism and modulation of flavonoid levels and lipid synthesis have been investigated in arbuscular mycorrhizal (AM) roots (Fester and others 2002; Schlie-mann and others 2008). The concentration of phenolics in *Echinacea purpurea* is also significantly increased upon AM colonization (Araim and others 2009).

Although knowledge of molecular interactive mechanisms regulating plant-endophyte associations is limited, it is also reasonable to assume that these intimate plant–fungal interactions are much more than simple physical contact. Similar to mycorrhizae, cell recognition and biochemical changes must be present (Mucciarelli and others 2002). In peppermint (*Mentha piperita*), a plant growth-promoting endophytic fungus HSF can change the composition of the essential oil and increase terpene volatilization from leaves. The authors contend that terpenoid profiling and distribution among roots, leaves, and fungal mycelia may be critical for establishing a fine-tuned plant–endophyte mutualistic association (Mucciarelli and others 2003, 2007). Likewise, endophyte-induced targeted plant secondary metabolite accumulation offers new tools for medicinal plant cultivation and breeding. Currently, *Piriformospora indica* and *Sebacina vermifera*, living as endophytes in a variety of hosts, have gained increasing attention. In vitro and ex vitro coculture systems verify that both fungal species possess pronounced plant growth-

Fig. 1 Procedures for clarification of the morphological, physiological, and molecular interactions in plant–fungal endophyte associations. REMI, restriction enzyme-mediated integration; ATMT, *Agrobacterium tumefaciens*-mediated transformation; PR protein, pathogenesis-related protein



stimulating capacity and induce plant secondary metabolite accumulation, such as spilanthol in *Spilanthes calva* and podophyllotoxins in *Linum album* (Rai and others 2001, 2004; Baldi and others 2008). In addition, *Fusarium mairei*, a paclitaxel-producing endophyte, also enhances the paclitaxel production in *Taxus cuspidate* cell suspension (Li and Tao 2009). These interesting achievements may motivate us to explore fungal endophytes as effective and beneficial fungal agents to enhance the chemical production of medicinal plants and develop novel strategies to improve the quality of plant-based medicine on an industrial scale (Saxena and others 2005; Yuan and others 2007).

Host-Improved Performance and Stress Tolerance Conferred by Endophytic Symbionts

Plant nutrition acquisition and growth promotion strengthened via endophytic symbionts resemble some

features of mycorrhizal interactions but clearly differ from mycorrhizal symbiosis in some aspects (Sherameti and others 2005; Kogel and Schafer 2009). Phytohormones, siderophores, and nitrogen- and phosphorus-assimilating related enzymes produced by endophytic fungi have been extensively determined and are strongly related to the improvement of plant nutrition and performance (Gasoni and others 1997; Maccheroni and Azevedo 1998; Bartholdy and others 2001; Obledo and others 2003; Malla and others 2004; Sheraemti and others 2005; Sirrenberg and others 2007; Khan and others 2009).

Plants are always exposed to adverse environments such as arid lands and high temperatures or to pathogens, but they routinely survive. Recent advances in fungal ecology have revealed that some of this tolerance or resistance comes not from the plant itself, but from the cryptic fungi residing in the plant's tissues. Although mycorrhizal fungi and grass clavicypitaceous endophyte-mediated stress resistance has been well documented (Pozo and

Azcon-Aguilar 2007; Kuldau and Bacon 2008), few details are known about the ecological significance of type II endophytes. In some ecosystems, DSEs live more frequently as dominant root mycobiota than do mycorrhizal fungi (Wilberforce and others 2002a, b), which is indicative of equally important functions of root endophytic fungi.

Recently, symbiotic functionality of foliar endophytes (Herre and others 2005), DSEs (Mandayam and Jumpponen 2005), and other opportunistic, avirulent root microsymbionts (Harman and others 2004; Bacon and Yates 2006) has begun to be revealed. Moreover, some type II endophytes are vertically transmitted and also generally increase the vigor and biomass of plants (Ernst and others 2003; Barrow and others 2004b; Lucero and others 2006). Interestingly, the host range of endophytic fungi recovered from natural vegetation or extreme habitats is usually extensive, suggesting future biotechnological applications of native functional fungal endophytes as inoculum by transferring them into nonhost important crop plants for improvement of desired agronomic traits (Barrow and others 2008; Lucero and others 2008; Maciá-Vicente and others 2008b).

There are three hypothetical explanations of how type II endophytes confer host resistance to a series of biotic and abiotic stressful conditions. “Habitat-adapted symbiosis” is when symbiotically mediated resistance is a habitat-specific phenomenon and at least some plants rely on intergenomic epigenetic mechanisms provided by fungal endophytes to combat extreme environments. Native fungal endophytes isolated from geothermal habitats conferred survival to plants under high temperature but not salt tolerance. Moreover, some endophytic fungal strains recovered from crops conferred disease resistance but not heat or salt resistance. “Symbiotic modulation” is when selective pressures in adjacent microhabitats drive plants to change the endophytic fungal community and endophytes change plant hosts; it may play an important role in plant invasion. “Symbiotic lifestyle switching” is when the same endophytic fungal species may express both mutualistic and pathogenic lifestyles depending on different host species (Rodríguez 2007; Rodríguez and others 2008). However, there would be an exception for “habitat-adapted symbiosis”. Surprisingly, we are still fascinated with the new champion of symbiosis, *Piriformospora indica*, which was isolated from the rhizosphere soil of the desert. Growth, yield, salt tolerance, and pathogen protection in a variety of plants are simultaneously promoted after inoculation with *P. indica* (Waller and others 2005, 2008). These results challenge the notion that pathogen resistance always has physiological costs resulting in yield decrease (Kharkwal and others 2008).

Nevertheless, possible cellular mechanisms of improved abiotic stress tolerance as a result of plant-endophyte mutualism seem scarcely understood. It has been presumed that fungal infection resulted in the priming of expression

of a set of stress-related genes or eliciting stress-hormone production (for example, abscisic acid) compared to uncolonized plants (Sherameti and others 2008). Furthermore, symbiotic fungi may also promote the activation by plants of the biosynthesis of proline or antioxidant enzymes to scavenge the ROS (Rodríguez and Redman 2005; Baltruschat and others 2008). Increased antioxidant enzyme activity, including CAT (catalase), APX (ascorbate peroxidase), DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase), and GR (glutathione reductase), plays a significant role in tolerance to abiotic stressors. Baltruschat and others (2008) point out that these enzyme activities are maintained at a high level in *P. indica*-infected plants but decrease gradually in uninfected plants. Furthermore, fungi may also produce compounds necessary for heat or drought tolerance. Limited evidence has indicated that the mucilaginous matrix (polysaccharide), melanin, mannitol, and trehalose produced by endophytic fungi are necessary for enabling hosts to work better (Barrow and others 2004a; Usuki and Narisawa 2007). Although many reports have verified the protective properties of trehalose against abiotic stress in bacteria, fungi, and animals, most plant species do not appear to accumulate detectable levels of trehalose (Garg and others 2002). Transgenic rice plants that overproduce *Escherichia coli* trehalose biosynthetic genes have high tolerance to abiotic stress (Garg and others 2002). It seems likely that production of trehalose in vivo by endophytes may be the key mechanism for allowing hosts to compete successfully under stress conditions. Moreover, ternary interactions with mycoviruses may also be an important component of plant–endophyte associations with respect to stress tolerance (Márquez and others 2007), indicating more complicated interactive patterns than in mycorrhizal symbiosis.

It is recently found that bacteria are intimately associated with some symbiotic fungi and this could be extended to *P. indica*, because bacteria of the species *Rhizobium radiobacter* (formerly *Agrobacterium tumefaciens*) are closely associated with this fungus (Sharma and others 2008). Specific fluorescence-labeled phylogenetic oligonucleotide probes for these bacteria and confocal laser scanning microscopy could clearly reveal the endofungal localization. In addition, Sharma and others hypothesize whether the endofungal bacterium contributes to the observed plant growth promotion and increased systemic resistance. Therefore, it is presumed that bacteria–fungus–plant or virus–fungus–plant triple symbiosis is highly required for the fulfillment of symbiotic functions.

It has long been recognized that exploitation of fungi as biological control agents is commercially valuable. Unfortunately, to date, the commercialization process and the market success of biological control agents have been limited. *Trichoderma*, *Gliocladium*, and some entomogenous

fungi are promising agents (Hjeljord and Tronsmo 1998; Vega and others 2008). Therefore, searching for novel and effective fungal organisms is urgently needed. Recently, emphasis on resistance to plant disease afforded by endophytic fungi is emerging and recorded. Unlike antagonistic fungi from soil, all of which are sensitive to environmental factors and to competition with other cocolonizing microbes, endophytes preferentially reside in inner plant tissues and are protected from the physical barrier and encounter less competition. Most importantly, fungal endophyte-mediated induced resistance in plants has also been addressed (Arnold and others 2003; Ganley and others 2008). Hence, it can be predicted that development of endophytic fungi as alternative microbial agents may be a long-term biocontrol strategy for plant protection and disease management (Redman and others 1999; Arnold and others 2003; Waller and others 2005).

Three potential mechanisms underlying endophyte-mediated resistance to herbivorous insects have been hypothesized and tested (Arnold and Lewis 2005; Arnold 2008). Direct antagonism and production of toxic secondary metabolites are recognized as general patterns for reducing insect performance (Johnson and Whitney 1994; McGee 2002; Miller and others 2002). Recently, some pathogens of insects—entomopathogenic fungi including *Beauveria bassiana*, *Paecilomyces* sp., *Clonostachys rosea*—were also characterized with their endophytism in living plant tissues (Quesada-Moraga and others 2006; Vega and others 2008). Irrigation, spraying, and injection inoculation methods confirm the wide host range of these fungal groups, which provides a model system for understanding the ecology of endophytic symbiosis and the roles of entomopathogenic fungi with regard to insects (Posada and others 2007). Finally, Carroll (1991) proposed a hypothesis that endophytes protect hosts in a mosaic-class defense manner whereby the diverse fungal endophytes establish a heterogeneous chemical background in leaves, resulting in different reactions to pathogens for genetically uniform plants.

Concluding Remarks and Perspectives

It is widely accepted that fungal and bacteria endophytes (Schulz and Boyle 2006) are ubiquitous in plants and are an important component of biological diversity on Earth. The abundance and diversity of fungal endophytes in plant tissues are accompanied by their diversified functions. Current knowledge of endophytic biology might be only the tip of the iceberg. Because nearly all plants participate in symbiosis with a variety of cryptic fungi, ignorance and underestimation of their entity may make it difficult to fully understand plant biology (Herre and others 2007). If fungal effects on hosts are clearly large, it is hard to conclude that

the plant phenotype (properties) is due to the individual plant (Moran 2007). The benefits and costs for accommodating endophytic fungi in plants may also become a challenging problem (Clay 2004), still yet to be elucidated.

Another interesting subject waiting to be clarified is how many more novel multifunctional symbiotic endophytes like *P. indica* can be discovered in natural habitats? With the unprecedented loss of biodiversity, it is imperative to collect endophyte germplasm, especially for rare and endangered plant species (Strobel 2007). Meanwhile, one can speculate that fungi isolated from unique or extreme environments are generally related to their molecular traits that affect plant performance (Kogel and Langen 2005), deserving further consideration and exploration. Bioprospecting “competent or true endophytes” for biological control of pathogens and plant breeding is an important issue for future research. Examination of the infectiveness, effectiveness, and competitiveness of recovered fungal isolates when establishing beneficial plant-microbe associations is a principle for bioprospecting (Hardoim and others 2008). Furthermore, increasing evidence indicates that *P. indica* displays clearly unique characteristics in interactions with plants when compared to AM fungi (Deshmukh and others 2006; Kogel and Schafer 2009). Therefore, deciphering host–endophyte associative symbiosis may generate a great deal of unexpected information about plant–fungal mutualistic interactions. *Muscodora* may also be a peculiar fungal genus. Although production of antimicrobial volatile compounds in vitro by these fungi has been determined, knowledge of the distribution pattern, ecology, and basic biology of *Muscodora* is still largely incomplete (Strobel 2006; Strobel and others 2007). The absence of the teleomorph state and any sporulating structure of *Muscodora* raises questions about their reproduction style and transmission model in nature.

Further exploitation of the ecological significance of horizontally transmitted endophytes will extend our views on plant–environment interactions and fungal ecology and lead to new strategies for plant cultivation and breeding.

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