Fungal endophytes and their interaction with plant pathogens

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Abstract
Endophytes are fungi which infect plants without causing symptoms. Fungi belonging to this group are ubiquitous, and plant species not associated to fungal endophytes are not known. In addition, there is a large biological diversity among endophytes, and it is not rare for some plant species to be hosts of more than one hundred different endophytic species. Different mechanisms of transmission, as well as symbiotic lifestyles occur among endophytic species. Latent pathogens seem to represent a relatively small proportion of endophytic assemblages, also composed by latent saprophytes and mutualistic species. Some endophytes are generalists, being able to infect a wide range of hosts, while others are specialists, limited to one or a few hosts. Endophytes are gaining attention as a subject for research and applications in Plant Pathology. This is because in some cases plants associated to endophytes have shown increased resistance to plant pathogens, particularly fungi and nematodes. Several possible mechanisms by which endophytes may interact with pathogens are discussed in this review.

Additional key words: biocontrol, biodiversity, symbiosis.

Resumen.
Revisión. Los hongos endofíticos y sus interacciones con patógenos de plantas

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Los hongos endofíticos infectan plantas sin causarles síntomas. Este tipo de hongos es omnipresente y hasta la fecha, no se conoce ninguna especie vegetal en la cual no se hayan detectado endofitos. La diversidad biológica de este grupo de hongos es enorme, sirva de ejemplo que en algunas especies de plantas se han identificado más de cien especies endofíticas. Dentro de este grupo existen distintos mecanismos de transmisión y estilos de vida simbiótica. Los patógenos latentes parecen representar un subgrupo relativamente pequeño dentro de las micobiotas endofíticas asociadas a especies vegetales, también compuestas por saprofitos latentes y mutualistas. Algunos endofitos son generalistas, capaces de infectar a numerosas especies vegetales, mientras que otros son especialistas que solo infectan a una o unas pocas especies. Los hongos endofíticos están recibiendo atención por parte del mundo de la Fitopatología debido a que en algunos casos se ha observado un aumento de la resistencia a patógenos debido a la presencia de endofitos. En esta revisión se discuten algunos mecanismos de interacción entre endofitos, plantas y patógenos.

Palabras clave adicionales: biodiversidad, control biológico, simbiosis.

Introduction

Plant pathogens and arbuscular mycorrhizae are the best known fungi associated to plants. In addition to those, numerous species of fungi known as endophytes inhabit the tissues of all plant species. These fungi live inside plant tissues without inducing apparent symptoms in their hosts.

The existence of fungi inside the organs of asymptomatic plants has been known since the end of the XIXth century (e.g. Guerin, 1898). However, except for a few sporadic works (e.g. Sampson, 1933), it is not until the end of the XXth century when fungal endophytes began to receive more attention from scientists. An important year in the history of endophyte research is 1977, when Charles Bacon and colleagues found the cause of fescue toxicosis, a
syndrome suffered by cattle fed in pastures of the grass *Festuca arundinacea* (Bacon et al., 1977). These researchers found that, although they showed no symptoms, most plants of *Festuca arundinacea* from pastures where cattle suffered intoxications, had their leaves and stems systemically colonized by a fungus. Afterwards, this fungus was identified as *Neotyphodium coenophialum* (Morgan-Jones and W. Gams) Glenn, C.W. Bacon and Hanlin (Fam. *Clavicipitaceae*), it was found that infected plants contained several toxic alkaloids, and that *Neotyphodium* species can be beneficial to their plant hosts, increasing their tolerance of biotic and abiotic stress factors (Schardl et al., 2004). Today, *Neotyphodium* species and their *Epichloë* teleomorphs constitute the best known and most intensively studied group of fungal endophytes.

However, *Neotyphodium* and *Epichloë* endophytes only represent a small fraction of the endophytic species associated to grasses. Some grass species are associated to more than a hundred different species of fungal endophytes (Sánchez Márquez et al., 2007), and this number can be much greater for members of other plant families (Arnold et al., 2000; Stone et al., 2004). Endophytes are ubiquitous in the plant world, no report of a plant species not associated to them is known. In addition, in a given plant species, individuals without endophytes are rare.

Endophytes are known to affect the interactions of plants with their environment, and to alter the course of their interactions with plant pathogens. In spite of this, fungal endophytes are still quite inconspicuous in plant pathology textbooks. This is likely to change soon. In addition to represent a source of organisms for disease control and plant improvement, the study of endophytes may have an important influence in the conceptual framework where plant-pathogen interactions are interpreted and investigated.

The objective of this review is to acquaint the reader with some general information about the characteristics of fungal endophytes, and in particular, to focus on those aspects of
endophyte research where interactions among endophytes and plant pathogens have been studied.

The species diversity of fungal endophytes

The procedures most commonly used for endophyte surveys are based on the surface disinfection of apparently healthy plant tissue samples to kill epiphytic fungi (Bills, 1996). The surface-disinfected plant samples are subsequently placed on a synthetic growth media and, when endophytic hyphae emerge from the plant tissue and start growing in the agar medium, isolations can be made. With this technique obligate biotrophs or fungi not growing well in the selected medium will not be isolated. As a result, the real number of endophytic species in a sample can be underestimated. Non culturable endophytes do exist, and fortunately some techniques allowing their detection in plant tissues have been developed (Neubert et al., 2006; Duong et al.; 2006; Gallery et al., 2007).

An average of about 50 endophytic species per plant species was found in surveys done before the year 2000 (Stone et al., 2004). When molecular methods for the identification of fungi began to be applied to endophyte research (i.e. rDNA sequencing; Arnold et al., 2000; Guo et al., 2000), the number of fungal species identified per host plant species increased substantially. The reason for this is that an important proportion of fungal isolates may be sterile in laboratory cultures. Genotypic identification methods have allowed to identify, or at least to distinguish, among sterile cultures.

When the results of endophyte surveys are analyzed in species accumulation curves it is often found that these curves are non-asymptotic (Fig. 1), suggesting that if more plant samples would have been analyzed, more endophytic species would have been discovered. These results imply that most surveys of endophytic mycobiota underestimate the real number of species associated to a given plant species. In fact, when mathematical estimators of the
total species abundance are applied to field data (Chazdon et al., 1998), the number of endophytic species potentially associated to a plant species is often estimated in several hundreds (Sánchez Márquez et al., 2007).

Endophyte assemblages are composed by rare or singleton species which are isolated only once or very few times, and by dominant or plural species which are frequently isolated from a given host species (e.g. Neubert et al., 2006). Singleton species are the main factor driving non-asymptotically species accumulation curves. When the singleton species found in a survey are excluded from the data used to plot a species accumulation curve, the resulting lines may be asymptotic (Fig.1). This suggests that the number of plural taxa commonly associated to a given plant species is much more limited than the group of singleton species which occasionally infect a plant.

Another factor contributing to the large diversity observed in endophytic assemblages is geographical variation, the taxa isolated from the same host species tends to change from one location to another (Collado et al., 1999). In a geoclimatic context, endophytic assemblages appear to be richer in tropical than in temperate or cold zones of the world. (Fisher et al., 1995; Arnold and Lutzoni, 2007). Plant age also has an effect upon endophyte diversity. As time of exposure to endophyte inoculum increases, plants seem to accumulate an increasing number of endophytes in their tissues. Because of this, older plant parts may harbour more endophytes than younger ones (e.g. Arnold et al., 2003).

It is thought that only a small proportion, about 5% of the existing fungal species are known (Hawksworth, 2001). As new environments are explored, new species will be found and classified. Endophytic fungi represent a very diverse group, and as new studies on this kind of fungi are made, it is very likely that progress in the discovery of new fungal species will be made.
Biological characteristics of endophytic species

Tissue colonization and specificity

Many endophytes locally infect plant parts, being restricted to a small tissue area. This is supported by the fact that often, several endophytic species are recovered from different fragments of the same plant. In contrast, *Neotyphodium* and *Epichloë* species systemically infect the intercellular space of leaves, reproductive stems, and seeds of their hosts (Fig. 2). These systemic endophytes can be isolated from multiple fragments of the same plant. Tissue and organ specificity also occurs, and some endophytes may be found in specific plant parts such as roots, leaves, or twigs, while others may infect several of these parts (Stone *et al.*, 2004).

Regarding the specificity shown by endophytes for their host plants, specialists as well as generalists do occur in this group. For example, *Neotyphodium* endophytes have a narrow host range, being confined to one or two plant species. Other endophytic fungi such as *Alternaria*, *Penicillium*, or *Piriformospora* have wide host ranges, encompassing species within different genera or plant families (Stone *et al.*, 2004; Waller *et al.*, 2005).

Symbiotic lifestyles

Different symbiotic lifestyles occur among endophytes. Depending on the species involved, the outcome of a plant-endophyte interaction can range from antagonism to mutualism. This is why the distinct range of plant-endophyte interactions has been referred as a continuum (Saikkonen *et al.*, 1998; Schulz and Boyle, 2005). Apparently healthy plants are the raw material used for endophyte surveys. Therefore, latent pathogens may be isolated from such plants if sampling is done before symptoms appear (Mostert *et al.*, 2000; Photita *et al.*, 2004). However, latent pathogens do not seem to constitute an important fraction of endophyte assemblages, most endophytes do not cause symptoms on plants. For example, out
of 109 different fungal species identified in *Dactylis glomerata* L, only 5 corresponded to known pathogens of that grass (Sánchez Márquez *et al.*, 2007).

Some saprophytic fungi commonly found in senescent plant parts have been isolated as endophytes from healthy tissues (Promputtha *et al.*, 2007). Such endophytic species behave as latent saprophytes, these fungi may be asymptomatic and spatially restricted while their hosts grow, but will grow unrestricted and reproduce when the infected host tissue senesces or dies.

At the other end of the continuum there are endophytes which are beneficial to their hosts, the best known in this group are the *Neotyphodium* and *Epichloë* species which can provide antiherbivore defense, as well as drought tolerance and improved nutrient use to their plant hosts (Schardl *et al.*, 2004). In addition to other mutualistic species known to benefit plants by protecting them against pathogens, endophytes such as *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer, G. Kost and P. Franken (Waller *et al.*, 2005), *Acremonium strictum* W. Gams (Hol *et al.*, 2007), and some *Stagonospora* species (Ernst *et al.*, 2003) can enhance the growth of their hosts.

**Transmission and acquisition of endophytes.**

Endophytes may infect plants by means of horizontal transmission, when their inoculum is transported to another plant, or vertically when they infect the seed progeny of an infected plant. Horizontal transmission seems to be the predominant mechanism of dispersion among endophytic species. Some studies have shown that seeds and seedlings are virtually endophyte-free, and the incidence of fungal endophytes increases as leaves or seeds grow older (Arnold *et al.*, 2003; Gallery *et al.*, 2007). This type of dynamics must be driven by horizontal transmission.
There is a paradox related to the understanding of the mechanisms of horizontal transmission of endophytes: for horizontal transmission to occur, endophytic inoculum has to be produced and dispersed. But in an asymptomatic host, where and when is the inoculum produced? In the case of latent saprophytes, the inoculum which infects new hosts could be produced when infected host tissue dies. Some saprophytes which produce fructifications in dead plant parts have been identified as endophytes in healthy tissues (Sánchez Márquez et al., 2007). From this point of view, leaf litter may be an important source of endophytic inoculum (Bills and Polishook, 1994). In other situations, inoculum may be produced in an inconspicuous way in infected hosts. For example, some grasses infected by Epichloë endophytes have a microscopic layer of hyphae and conidia in the surface of their leaves. It is thought that this inoculum might horizontally infect new hosts (Tadych et al., 2007). Phytophagous insects may also take part in the spread of endophytes, since spores of some fungal species are resistant to gut digestion, and are present in their fecal pellets (Devarajan and Suryanarayanan, 2006).

Vertically transmitted endophytes are discovered by means of studying seed transmitted fungi, and such studies are scarce (Gallery et al., 2007). Neotyphodium endophytes and some Epichloë species (e.g. E. festucae Leuchtm., Schardl and M.R. Siegel, E. sylvatica Leuchtm. and Schardl) are vertically transmitted to host progeny by means of seed infection. Close to 100% of the seeds produced by an infected plant contain fungal mycelium near the embryo and in the aleurone layer (Fig. 2B). These seeds will give rise to asymptomatic infected plants. Therefore, these endophytic species are vertically transmitted in a fashion similar to a maternally inherited character (Schardl et al., 2004). Because of this, the incidence of these endophytes is very high in natural populations of their hosts (Arroyo Garcia et al., 2002).
How fungal endophytes can affect plant disease

Evidence showing that endophytes have a role in the outcome of plant–pathogen interactions leading to disease has been increasing in recent years. Diverse mechanisms by which they may counteract pathogen development have been observed. For example, some endophytic species may induce plant defense mechanisms which counteract pathogen attack, others produce antibiotic substances which inhibit pathogen growth, competition for plant space and resources may also occur between resident endophytes and incoming pathogens; finally, some parasites of plant pathogens are known to behave as endophytes.

Interactions with plant pathogenic fungi.

Many endophytic species produce antibiotic substances (Schulz and Boyle, 2005; Strobel, 2002; Wang et al., 2007). Liquid extracts from endophyte cultures have been found to inhibit the growth of several species of plant pathogenic fungi (Liu et al., 2001; Park et al., 2005; Inácio et al., 2006; Kim et al., 2007). If such compounds where produced by endophytes in planta, this could constitute a defense mechanism against fungal pathogens.

Experiments where plant protection against pathogenic fungi is observed after the inoculation of plants with endophytes, as well as after the application of endophytic culture filtrates, suggest that the endophyte may produce an antifungal compound or a substance that induces plant defense mechanisms in the plant. This is the case with Chaetomium and Phoma endophytes of wheat, when these fungi were previously inoculated in plants, reduced severity of foliar disease caused by Puccinia and Pyrenophora spp. was observed and, the same protective effect was observed when only endophytic culture filtrates were applied to the plants (Dingle and McGee, 2003; Istifadah and McGee, 2006). In these experiments the effects of culture filtrates upon plant pathogens were not tested.

When a mixture of six species of endophytes frequently isolated from cacao (Theobroma cacao L.) trees was used to inoculate leaves of endophyte-free seedlings of this
plant species, the severity of a leaf disease caused by a Phytophthora sp. was significantly reduced in endophyte-inoculated leaves. A mechanism of induced plant resistance did not seem to be involved, because differences in disease severity were observed between endophyte-inoculated and non-inoculated leaves of the same plant. In this case, the protection against a pathogen could be the result of direct competition among endophytes already present in leaves and the pathogen (Arnold et al., 2003). For instance, most tissue available for infection may be already occupied, or endophytes may produce zones of inhibition restricting the entry of other fungi.

Endophyte infection may alter plant biochemistry in a way that defense mechanisms against pathogens are induced. Piriformospora indica Sav. Verma, Aj. Varma, Rexer, G. Kost and P. Franken is a root endophyte with a wide host range, including several species of cereals and Arabidopsis. Barley plants inoculated with this endophyte have shown resistance to a vascular (Fusarium culmorum (W.G. Sm.) Sacc.) and a leaf pathogen (Blumeria graminis (DC.) Speer), in addition to an increase in yield and salt stress tolerance (Waller et al., 2005). The protection against the leaf pathogen appears to be mediated by a mechanism of induced resistance, because in the pathogen-inoculated plants there is a defense response involving the death of host cells.

Some endophytes may be mycoparasites. Acremonium strictum W. Gams is an endophyte which has been frequently isolated from Dactylis glomerata L. and other grasses (Sánchez Márquez et al., 2007); recently it has been shown that this fungus is a mycoparasite of Helminthosporium solani Durieu and Mont., a potato pathogen (Rivera Varas et al., 2007).

A significant increase in resistance to dollar spot disease, caused by Sclerotinia homoeocarpa F.T. Benn., has been observed in Festuca rubra L. cultivars infected by Epichloë festucae Leuchtm., Schardl and M.R. Siegel. (Clarke et al., 2006). Cultivars of several turfgrass species infected by Epichloë and Neotyphodium endophytes are
commercially available at the present time. The efficient vertical transmission of these endophytes has allowed the production of infected seed at a commercial scale. Since *Neotyphodium* and *Epichloë* infected cultivars have shown increased resistance to herbivores, plant pathogens, and some conditions of abiotic stress, the use of such symbiotic cultivars can result in a reduction in the use of insecticides and fungicides in lawns (Brilman, 2005). Similar applications of other species of endophytes may be seen in the future.

The above studies suggest that the outcome of some pathogen attacks may be dependent on the endophytic mycobiota associated to a host plant. Therefore, the endophytic assemblage of a given species may represent a source of organisms with potential applications for disease control in the same plant species.

Out of the multiple species that can penetrate and infect a plant, only a relatively small subset, that of the pathogens, produces disease. This shows that part of the plant disease cycle is shared by pathogens of endophytes. Once a fungus enters a plant it can behave as an endophyte or as a pathogen, and it seems that a majority of plant associated fungi act as endophytes. What is the difference between infection processes caused by endophytes and pathogens, is a good question for plant pathologists. Some studies directed to answer such question point out to fungal as well as to host characteristics. For instance, a mutation in a single locus can convert a pathogen such as *Colletotrichum magna* Jenkins and Winstead in a mutualistic endophyte (Freeman and Rodriguez, 1993). However, some isolates of the above species may behave as a pathogen in cucurbits or as an endophyte in some species of other plant families (Redman *et al.*, 2001).

**Interactions with nematodes**

Inhibitory effects against some species of migratory and sedentary endoparasites occur in grasses infected by *Neotyphodium* endophytes (West *et al.*, 1988; Kimmons *et al.*, 1990).
Neotyphodium species infect aerial tissues, not roots. Therefore, the inhibitory effects observed in infected plants were interpreted as the result of fungal alkaloids being translocated to roots. This was supported by the fact that some naturally occurring Neotyphodium strains deficient in the production of ergot alkaloids do not show protective effects as good against Pratylenchus sp. as those observed in ergot alkaloid producing strains (Timper et al., 2005). In contrast, other experiments showed that the amount of ergot alkaloids translocated to roots is very small, and experiments with Neotyphodium knockout mutants having their pathway of ergot alkaloid synthesis disrupted suggested that these alkaloids are not responsible for the inhibition of nematode populations in endophyte infected plants (Panaccione et al., 2006). Nevertheless, other types of alkaloids with antiherbivore activity are produced by Neotyphodium species, and chemical changes such as the production of phenolic compounds do occur in Neotyphodium-infected roots (Malinowski and Belesky, 2000). In conclusion, Neotyphodium endophytes provide host plants with protection against several nematode species, but the mechanism of action underlying this process is still unknown.

Non pathogenic strains of Fusarium oxysporum E.F. Sm. and Swingle isolated from plant roots are other group of endophytes known to be implicated in antinematode activity. Culture filtrates of F. oxysporum have an inhibitory effect on Meloidogyne incognita Kofoid and White, suggesting that fungal toxins could be the mechanism of interaction. (Hallmann and Sikora, 1996). However, the mechanism of Fusarium inhibition of nematodes appears to be more complex than a toxin-operated system. In an experimental setup where banana plants were grown in a split root system, the plants were resistant to Radopholus similis Cobb, Thorne in the root half which was not inoculated with a Fusarium endophyte. In this case, a phenomenon of systemic plant resistance induced by the endophyte appeared to be the mechanism of resistance to the nematode pathogen (Vu et al., 2006).
Another type of plant protection mediated by endophytic fungi may come from nematophagous fungi which can inhabit plant roots as endophytes (Bordallo et al., 2002). In a similar fashion, some species of entomophagous fungi (e.g. Beauveria bassiana (Bals.-Criv.) Vuill., Torrubiella confragosa Mains, Metarhizium anisopliae (Metschn.) Sorokin) have been isolated from several host plants, and appears that part of their life cycle can be endophytic (Bills, 1996).

In conclusion, it is very likely that fungal endophytes affect the outcome of nematode attacks in plants, and certain endophytes could be used for nematode protection in agriculture.

**Interactions with bacteria and viruses.**

Tests of the influence of endophytes upon bacterial and viral pathogens are not as numerous as those made with other plant pathogens. Bactericidal effects of endophyte culture extracts have been demonstrated and do not seem to differ from those observed for fungi or nematodes (Wang et al., 2007).

In the case of viruses, the incidence of barley yellow dwarf virus (BYDV) was lower in *Lolium pratense* infected by *Neotyphodium* than in endophyte free plants. Since BYDV is transmitted by means of aphid vectors, toxic fungal alkaloids may be the reason for this effect, in fact, aphid reproduction was lower in endophyte infected plants than in those free of endophyte (Lehtonen et al., 2006).

A very interesting connection of a different kind exists among endophytes and viruses. A *Curvularia* endophyte of the plant *Dichanthelium lanuginosum* (Elliott) Gould was found to confer tolerance to high soil temperatures to the plant. Further observation of this system led to the discovery that a virus infecting the endophyte was an important factor contributing to the heat tolerance observed in the plants. Furthermore, the virus-infected endophyte could be used to confer heat tolerance to tomato plants (Marquez et al., 2007). *Epichloë festucae* virus
1 (EfV1) is another virus which asymptotically infects the grass endophyte *Epichloë festucae*, in this case it is not known if the presence of the virus in the endophyte affects the plant host (Romo *et al*., 2007).

**Conclusions**

The inner space of plants represents an ecological niche where numerous species of fungal endophytes live. These ubiquitous fungi have an affect in the way plants interact with their environment. In some ways, a plant is a plant and its endophytes. It appears that most endophytic assemblages contain mutualistic species which help plants to cope with biotic and abiotic stress situations. Because of this, endophytes may have an important role in the adaptation of plants to some particular environments (Rodriguez *et al*., 2004). In addition, they represent a group of organisms with a very good potential for applications in plant improvement and disease control. Some examples of this are already available in the market (i.e. *Neotyphodium* and *Epichlöe* infected turf and forage cultivars of some grasses), other applications in this line are likely to appear in the future.

Multiple endophytic species are capable of penetrating and infecting plants without inducing symptoms. This suggests that the penetration of plant tissues is not a process particularly associated to pathogenesis. In this sense, the conceptual framework provided by the comparative study of plant pathogens and endophytes may contribute some interesting questions and answers to the science of Plant Pathology.

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Figure 1. Species accumulation curves show the relationship between the number of plant samples analyzed and the number of different endophytic species found. Non-asymptotic curves (continuous line) are often observed in endophyte surveys. Such curves suggest that increasing the number of plant samples analyzed would lead to the discovery of more endophytic species. An accumulation curve would become asymptotic when all endophytic species associated to a plant species have been collected. Asymptotic curves can also be observed when only plural species which have been isolated from more than one plant are considered (dotted line). These curves suggest that the most common endophytes associated
to a plant species represent a limited set, and it is the singleton species which only
occasionally infect plants the ones which drive curves non-asymptotically. The data shown is
from a survey of endophytes in the grass *Dactylis glomerata* (Sánchez Márquez *et al.*, 2007).

Figure 2. Light micrographs showing hyphae (indicated by triangles) of the endophytic
fungus *Neotyphodium coenophialum* in the intercellular space of a leaf (A), and in the
aleurone layer of an infected seed (B) of *Festuca arundinacea*. Plants infected by this
endophyte are asymptomatic and most seeds produced by an infected plant are infected by the
fungus, which is vertically transmitted.