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Non-systemic fungal endophytes of grasses

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ABSTRACT

Many fungi behave as endophytes in grasses. Unlike the well known *Epichloë*/*Neotyphodium* species, most other endophytes are not capable of systemic colonization of plant organs, or seed transmission. The species diversity of the non-systemic endophytic mycobiota of grasses is large, dominated by ascomycetes. The relative abundance of species is very unequal, a few dominant taxa like *Acremonium*, *Alternaria*, *Cladosporium*, *Epicoccum* and *Penicillium* spp., occur in many grasses and locations. In contrast, many rare species are isolated only once in endophyte surveys. The possible ecological functions of endophytes are diverse, and often unknown. Latent pathogens represent a small fraction of endophytic mycobiotas, indicating that many non-pathogenic fungal taxa are able to enter plants overriding defence reactions. Some dominant species behave as latent saprotrophs, sporulating when the host tissue dies. Endofungal viruses and bacteria occur among endophytic species, but their effect in their hosts is largely unknown.

Introduction

After *Neotyphodium* species were found to be responsible for the toxicity of pasture grasses used for animal production (Bacon *et al.* 1977; Fletcher & Harvey 1981), surveys of endophytes in other plant families showed that this group of fungi and their *Epichloë* teleomorphs are exclusive to a small number of grass species. However, these studies further revealed the existence of a large number of fungal species capable of infecting plants without causing symptoms, and at the present time, no endophyte-free plant species has been reported (Stone *et al.* 2004; Arnold 2007; Sieber 2007; Hyde & Soytong 2008).

Depending on the extent of plant colonization and transmission mechanisms, several schemes for classifying endophytic species have been proposed (e.g., White 1988; Saikkonen *et al.* 1998; Schulz & Boyle 2005; Rodriguez *et al.* 2009). Interactions between *Epichloë* and *Neotyphodium* species and grasses, sometimes referred as systemic, clavicipitaceous, balansiaceous, type 1, or epichloë endophytes, constitute well-studied and documented examples of plant–fungal symbioses (Clay & Schardl 2002; Schardl *et al.* 2004; Kuldau & Bacon 2008; Rodriguez *et al.* 2009). These endophytes are mutualistic, colonize the intercellular space of leaves and stems in a systemic manner, and are vertically transmitted by seed. In contrast, little is known about

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symbiotic interactions in a large number of other endophytic species which appear to be non-systemic, not vertically transmitted, and have been reported in wild and cultivated cereals and grasses. Although there are some examples of non-clavicipitaceous endophytes that systemically infect their hosts (i.e. *Fusarium verticillioides*; Bacon & Hinton 1996), in this article we will use the name "non-systemic endophytes" to refer to all those endophytic species which do not belong to the *Epichloë* or *Neotyphodium* genera. Our objective is to review and synthesize some of the current knowledge about the diversity and characteristics of non-systemic endophytic species associated with grasses.

Incidence, species richness and abundance in assemblages of endophytes

Many surveys have shown that most or all individuals of a grass species are infected by fungi causing visible external symptoms and by non-systemic endophytes (Sprague 1950; Peláez *et al.* 1998; Wirsel *et al.* 2001; Sánchez Márquez *et al.* 2007, 2008, 2010; Porras Alfaro *et al.* 2008; Higgins *et al.* in press). However, in habitats inhospitable for plant or fungal life, like polar ecosystems, the incidence of endophyte infections seems to be relatively lower in grasses and in other plants (Arnold & Lutzoni 2007; Rosa *et al.* 2009). Plants in environments where exposure to aerial fungal inoculum is reduced (i.e. indoors) may also show a lowered incidence of endophyte infections.

The results of endophyte surveys depend on the methods of fungal detection or isolation used. Until the recent application of direct sampling of host plant DNA and amplification of fungal marker sequences (i.e. Vandenkoornhuyse *et al.* 2002; Neubert *et al.* 2006; Porras Alfaro *et al.* 2008; Higgins *et al.* in press), most surveys have been designed to detect species diversity *via* culturing, and this generally precludes the detection of unculturable species, such as obligate parasites, e.g., rusts, powdery mildews. Also, the culture media and dissection protocols used in a particular study may exclude some culturable fungi, and fast-growing fungi may be over represented (Bills & Polishook 1991; Hyde & Soytong 2008).

The endophytic mycobiota associated with a given grass species usually consists of a relatively large number of fungal species (Table 1; Supplementary Table I). A very unequal distribution of isolate richness among fungal species is typical of endophyte surveys (Fig 1). A few dominant endophytic taxa are commonly found in different plants and locations. In temperate grasses, among the group of dominant endophytic taxa there are *Alternaria*, *Acremonium*, *Cladosporium*, *Penicillium*, *Epicoccum* and *Aureobasidium* spp. (Table 1; Supplementary Table I). Dominant species of temperate and tropical grasses seem to have low host specificity, because they occur on multiple grass species, as well as in non-grass hosts (Stone *et al.* 2004; White & Backhouse 2007; Porras Alfaro *et al.* 2008; Sánchez Márquez *et al.* 2008; Higgins *et al.* in press; Khidir *et al.* 2010).

At the other extreme of the species abundance distribution in a survey, there are rare species that are represented by single isolates (Fig 1). These fungi are found only occasionally in a given plant species. Such interactions between plants and rare species possibly only occur when a given plant and fungal phenotype are confronted and may represent unstable associations. Such rare species often constitute more than half of the endophytic species identified in a survey (Neubert *et al.* 2006; Sánchez Márquez *et al.* 2007, 2008, 2010; Herrera *et al.* 2010; Higgins *et al.* in press) (Supplementary Table I). The existence of a large number of rare species in endophyte assemblages complicates the detection of host specific endophytes. Only species isolated from several individuals and

Table 1 – Species richness and dominant taxa in surveys of the non-systemic endophytic mycobiota in several grass species

Grass	Number of species	Dominant taxa[a]	Reference
Ammophila arenaria	75	*Alternaria*, unknown sp., *Podospora*	Sánchez Márquez *et al.* 2008
Bamboo *spp.*	60	Xylariales	Morakotkarn *et al.* 2007
Botriochloa macra	65	*Alternaria*, *Periconia*, *Phoma*	White & Backhouse 2007
Bouteloua gracilis	51	Pleosporales, Sordariales, Agaricales	Porras Alfaro *et al.* 2008
Dactylis glomerata	109	*Cladosporium*, *Helgardia*, *Acremonium*	Sánchez Márquez *et al.* 2007
Deschampsia antarctica	5	*Alternaria*, *Phaeosphaeria*, unidentified spp.	Rosa *et al.* 2009
Elymus farctus	54	*Alternaria*, *Podospora*, *Acremonium*	Sánchez Márquez *et al.* 2008
Festuca arizonica	14	*Neotyphodium*, unidentified spp.	Schultess & Faeth 1998
Holcus lanatus	134	*Alternaria*, *Cladosporium*, *Penicillium*	Sánchez Márquez *et al.* 2010
Hyparrhenia hirta	57	*Nigrospora*, *Periconia*, *Alternaria*	White & Backhouse 2007
Oryza	>11	*Alternaria*, *Cladosporium*, *Epicoccum*	Fisher & Petrini 1992
Phragmites australis	32	*Alternaria*, *Sporormiella*, *Rhizoctonia*	Peláez *et al.*, 1998
Phragmites australis	>20	*Microdochium*, *Cladosporium*, *Trichoderma*	Wirsel *et al.* 2001
Stipa grandis	34	*Pyrenopora*, *Alternaria*, *Phialophora*	Su *et al.* 2010
Stipa tenacissima	38	*Alternaria*, *Sporormiella*, *Rhizoctonia*	Peláez *et al.* 1998
Triticum aestivum	213	*Alternaria*, *Epicoccum*, *Idriella*	Sieber *et al.* 1988
Zea	23	*Alternaria*, *Aureobasidium*, *Acremonium*	Fisher *et al.* 1992
11 tropical grasses	94–124	Xylariales, Halosphaeriales, Phyllachlorales	Higgins *et al.* in press

a Three most abundant taxa.