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Diversity and host preference of fungi co-inhabiting *Cenococcum* mycorrhizae

*Gavin KERNAGHAN**, *Glenn PATRIQUIN*

Biology Department, Mount St. Vincent University, 166 Bedford Hwy., Halifax, NS B3M 2J6, Canada

ARTICLE INFO

Article history:
Received 1 December 2014
Revision received 30 March 2015
Accepted 3 April 2015
Available online 2 July 2015
Corresponding editor:
Kabir G Peay

Keywords:
Boreal forest
Dark septate endophytes
Ectomycorrhizae
Fungal communities
Fungal diversity
Helotiales
Root endophytes

ABSTRACT

Diverse fungal assemblages colonize the fine feeder roots of woody plants, including mycorrhizal fungi, fungal root endophytes and soil saprotrophs. The fungi co-inhabiting *Cenococcum geophilum* ectomycorrhizae (ECM) of *Abies balsamea*, *Betula papyrifera* and *Picea glauca* were studied at two boreal forest sites in Eastern Canada by direct PCR of ITS rDNA. 50 non-*Cenococcum* fungal sequence types were detected, including several potentially mycorrhizal species as well as fungal root endophytes. Non-melanized ascomycetes dominated, in contrast to the dark septate endophytes (DSE) reported in most culture dependent studies. The results demonstrate significant differences in root associated fungal assemblages among the host species studied. Fungal diversity was also host dependent, with *P. glauca* roots supporting a more diverse community than *A. balsamea*. Differences in root associated fungal communities may well influence ecological interactions among host plant species.

Introduction

Ectomycorrhizae (ECM) consist of both fine root and fungal tissues, resulting in a unique and metabolically active habitat within the soil ecosystem. A healthy ECM can support a wide variety of soil organisms, including a diverse array of fungi other than the dominant ectomycorrhizal symbiont (Bergero et al., 2000; Kernaghan et al., 2003; Menkis et al., 2005; Bergemann and Garbelotto, 2006; Morris et al., 2008; Urban et al., 2008; Wright et al., 2009). These secondary fungi may be other mycorrhizal species within the root (Cázares and Trappe, 1993; Olsson et al., 2000; Morris et al., 2008; Toju et al., 2014), or saprobes on the surface and associated mycorrhizosphere (Foster and Marks, 1967; Fogel, 1988). Ectomycorrhizae also harbor a wide range of asymptomatic fungal endophytes, which colonize the root tissue internally and appear to be as common as mycorrhizal fungi (Mandyam and Jumpponen, 2005; Weishampel and Bedford, 2006; Toju et al., 2013a).

Fungal endophytes can be found colonizing all types of plant tissue (Rodriguez et al., 2009), but the species assemblages associated with roots appear distinct from those colonizing shoots and leaves (Addy et al., 2005; Summerbell, 2005). Although the ecological role of these fungi is unclear (Mandyam and Jumpponen, 2005; Mayerhofer et al., 2013), some may be latent pathogens (Schulz et al., 1999) or saprotrophs (Kernaghan, 2013). Others may provide protection from soil pathogens (Narisawa et al., 2004), or improve plant growth

* *Corresponding author*. Tel.: +1 902 457 6328; fax: +1 902 457 6455.
E-mail address: gavin.kernaghan@msvu.ca (G. Kernaghan).
http://dx.doi.org/10.1016/j.funeco.2015.05.001

through phytohormone production (Khan et al., 2012), drought tolerance (Barrow, 2003), or improved access to soil phosphorus (Barrow and Osuna, 2002) or nitrogen (Upson et al., 2009).

Unlike mycorrhizal fungi, fungal root endophytes lack highly evolved absorptive structures involved in carbon and nutrient exchange (Brundrett, 2006), instead forming structures such as microsclerotia and hyphal coils within the host root (O'Dell et al., 1993; Vohník et al., 2003). Also, ECM fungal hyphae explore the soil environment and generally colonize fine roots only, while fungal root endophytes are more likely to be restricted to the roots, although they may grow throughout the root system (Menkis et al., 2004; Rodriguez et al., 2009; Toju et al., 2013a).

Much of the research into fungal root endophytes has focused on the dark septate endophytes, or DSE (Ahlich and Sieber, 1996; Grünig et al., 2002; Alberton et al., 2010; Newsham, 2011); a morphological classification encompassing fungal root endophytes with highly melanized hyphae. Although DSE are common, woody roots and ECM are colonized by a much more diverse array of fungi, including many non-melanized species, as demonstrated by several culture dependent (Schild et al., 1988; Fisher et al., 1991; Girlanda and Luppi-Mosca, 1995; Girlanda et al., 2002; Hoff et al., 2004; Summerbell, 2005; Kernaghan and Patriquin, 2011), and culture independent studies (Kernaghan et al., 2003; Kwaśna et al., 2008; Urban et al., 2008; Tedersoo et al., 2009; Izumi and Finlay, 2011; Toju et al., 2013a).

Recent evidence also indicates that some fungal root endophytes exhibit preference for particular host plants (Grünig et al., 2008; Tedersoo et al., 2009; Kernaghan and Patriquin, 2011; Quilliam and Jones, 2012; Tejesvi et al., 2013), although examples of specificity at levels seen in some ECM fungi are lacking (Molina et al., 1992; Toju et al., 2013a). There is also increasing evidence that some fungal root endophytes prefer ECM formed by particular species of ectomycorrhizal fungi (Urban et al., 2008; Tedersoo et al., 2009; Izumi and Finlay, 2011; Yamamoto et al., 2014), potentially representing a second level of root endophyte selection.

The occurrence of host preference, in combination with the range of possible effects that root associated fungi may have on their host plants, has motivated our research into differences in root associated fungal communities among host trees. In a previous study (Kernaghan and Patriquin, 2011), we investigated species composition, diversity and host preference in fungal root endophyte communities of ECM of boreal trees using culture-based techniques. To focus on differences among host plant species, and to avoid the potential influence of preference for ectomycorrhizal symbionts, ECM formed by *Cenococcum geophilum* (*Cg*) were focused on. *Cg* forms distinctive ECM that often dominate forest soils. It is widely dispersed, with a very broad host range and a relatively even distribution across soil horizons and successional stages (LoBuglio, 1999; Dickie et al., 2002). Although genetic variability in *Cenococcum* appears to be quite high (Douhan and Rizzo, 2005), the *Cg* ECM of a given host plant species should still represent a relatively homogeneous set of habitats for the study of root associated fungi.

Here, we revisit questions regarding the species composition, diversity and host preference of fungi associated with the *Cg* ECM of boreal trees, using direct PCR and cloning to eliminate the biases inherent in culture-dependant studies. We expected that the diversity of fungi associated with *Cg* ECM was underestimated in our previous work (Kernaghan and Patriquin, 2011) due to fast growing fungi such as *Phialocephala fortinii* outcompeting slower growing species. We also expected that a culture-independent approach would reveal obligate root associates that are not readily amenable to culture.

Materials and methods

Sampling

ECM samples were collected from two boreal forest sites; Mount Mackenzie, Cape Breton Highlands National Park, Nova Scotia and the Lac Duparquet Teaching and Research Forest, Abitibi-Témiscamingue, Québec. The sites are 1 400 km apart and support similar mixtures of mature *Abies balsamea*, *Betula papyrifera* and *Picea glauca* with sparse understory vegetation dominated by herbaceous species including *Cornus canadensis* and *Clintonia borealis*, as well as ferns such as *Osmundastrum cinnamomeum*. Ericaceous shrubs including *Kalmia* and *Vaccinium* spp. are also present but not dominant. More detailed site descriptions are given in Kernaghan and Patriquin (2011). At each site, four 2 m^2 sampling plots were established approximately 50 m apart. Each plot supported all three dominant tree species (*A. balsamea*, *B. papyrifera* and *P. glauca*; at least one of each per plot). During the snow free period between May and Sep., root systems were traced from the base of one individual of each of the three tree species on each plot (four root systems per tree species per site, for a total of 24 root systems) to the fine roots and associated ECM.

Dominant ECM types were identified on the basis of morphology, and included *C. g.*, *Laccaria bicolor*, *Tomentella* spp., *Russula* spp. *Tylospora* sp., "*Piceirhiza bicolorata*" and *Cortinarius* spp. Twenty healthy looking *Cg* ECM were collected from each root system, surface sterilized in 15 % hydrogen peroxide and frozen at −20° in AP1 extraction buffer (Qiagen) prior to DNA extraction.

DNA extraction and PCR

Genomic DNA was extracted from each set of 20 ECM using the DNeasy Plant Mini kit (Qiagen). ECM frozen in AP1 buffer were ground in a ceramic mortar and incubated at 65 °C for 30 min prior to following the manufacturer's instructions. Fungal ITS rDNA was amplified from the extracted genomic DNA using the fungal specific primers ITS-1F (Gardes and Bruns, 1993) and ITS-4 (White et al., 1990) in 25 μl reactions containing 2.5 U taq polymerase (New England Biolabs), 2.5 μl 10X PCR buffer (Promega) (0.05 M KCl, 0.01 M Tris—HCl, 0.01 % Triton X-100), 2.5 μM of each primer, 2.5 mM $MgCl_2$ and 0.2 mM dNTPs. In cases of PCR failure, reactions were amplified under the same conditions, but using GoTaq® master mix (Promega). Cycling parameters were as described in Kernaghan and Patriquin (2011).