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Review article

Genetic and Functional Diversity of Ericoid Mycorrhizal Fungi

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Abstract

Ericaceous plants are widespread on the globe and colonize substrates ranging from arid sandy soils to moist mor humus substrates. These plants also grow on soils polluted with metal ions, where toxicity is alleviated by the mycorrhizal fungal symbiont. A crucial point of current research on ericoid fungi is to understand whether this variety of environments corresponds to functional and genetic diversity of the associated fungal symbionts. Interesting features of ericoid mycorrhiza have derived from the genetic analysis of several fungal isolates: increased knowledge on their diversity has revealed that ericoid mycorrhiza can be very promiscuous, with multiple occupancy of the thin roots of ericaceous plants. Genetic diversity is also increased by the presence of several Group I introns in the nuclear 18S rDNA of most ericoid isolates, a feature rarely reported in eukaryotes. Biochemical analysis of hydrolytic enzymes produced by fungi from different environments also revealed diversity among isolates growing in polluted and non polluted soils. These results indicate that ericoid mycorrhizal fungi constitute a diverse population, both genetically and functionally.

Keywords: Ericoid fungi, *Hymenoscyphus ericae*, *Oidiodendron*, biodiversity, molecular techniques

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1. Introduction

Ericaceous plants are able to associate symbiotically with soil fungi to form a distinctive type of mycorrhiza, termed ericoid mycorrhiza (Perotto et al., 1995). The morphology of ericoid mycorrhiza is highly conserved even though different fungal and plant partners may associate in a range of combinations. The epidermal cells of the thin ericoid mycorrhizal roots are found to harbour dense coils of fungal mycelium, which remain enclosed within a single root cell without further spreading along the root tissues (Bonfante and Perotto, 1988; Perotto et al., 1995).

In nature, mycorrhizal ericaceous plants colonize very diverse environments. They occur as dominant species in heathlands, but they also associate with other endo- and ectomycorrhizal plants as understorey vegetation. Plants belonging to this group grow in areas ranging from the artic tundra to the Mediterranean area, on acidic as well as on calcareous soil. The ecological significance of ericoid mycorrhiza has been studied in more detail for low-mineral, acidic organic soils (Read, 1991 and references therein), where a crucial role in plant nutrition has been ascribed to the saprotrophic capabilities of the mycorrhizal endophyte. Ericoid mycorrhizal fungi can be isolated from infected roots and grown in axenic culture. The enzymatic abilities of these fungi, as tested in pure culture, indicate that they are well adapted to degrade the complex organic components found in humus soils (Leake and Read, 1991). Experimental evidence also demonstrated that symbiosis can make ericoid mycorrhizal plants successful in surviving high concentrations of toxic metal ions (Bradley et al., 1981).

Because ericaceous plants occur in a wide range of habitats and soil conditions, a high degree of diversity may be expected in the genetic and physiological abilities of the mycorrhizal fungal endophytes. A number of fungal species have been recorded as mycorrhizal on ericaceous plants, but relatively little is known about their genetic variability. The aim of this paper is to review the current knowledge of the genetic diversity of ericoid fungi and to provide some novel information on the organization of the nuclear ribosomal genes in different isolates. Some aspects of the functional diversity of ericoid fungi will also be discussed, in particular concerning the ability of some strains of *Oidiodendron* to tolerate conditions of heavy pollution.

2. Genetic Diversity of Ericoid Mycorrhizal Fungi

The ericoid fungal endophytes isolated so far belong to ascomycetes, although basidiomycetes have been observed by electron microscopy inside

naturally colonized roots (Bonfante, 1980; Peterson et al., 1980). Our knowledge on the extent of biodiversity of ericoid mycorrhizal fungi has been increasing rapidly in the recent years (Stoyke et al., 1992; Perotto et al., 1995). The first – and for about ten years the only – ericoid fungal endophyte described was an ascomycete with a dark, slow growing sterile mycelium, later identified by Read (1974) as *Hymenoscyphus ericae* (= *Pezizella ericae*). More recently, species of *Oidiodendron* were described as symbionts of ericaceous plants in Canada (Couture et al. 1983; Dalpè, 1986) and Europe (Douglas et al., 1989; Perotto et al., 1996). Many other fungal endophytes have been isolated from infected roots of Ericaceae (Stoyke and Currah, 1991) and Epacridaceae (Hutton et al 1993). However, the taxonomic position of most isolates is unknown because they do not form reproductive structures under the culture conditions tested.

Investigations on the genetic diversity of ericoid fungi has greatly benefited from PCR techniques as a mean to overcome the difficulties of morphological identification. PCR-RFLP analysis of different regions of the ribosomal genes has been used to investigate the identity and diversity of ericoid fungi. Examination of the small subunit of the ribosomal genes of the hyphomycete *Scytalidium vaccinii* revealed its close taxonomic relationship with *H. ericae* (Egger and Siegler, 1993), while RFLP analysis of the ITS region amplified from mycelia colonizing *Calluna vulgaris* roots has demonstrated that the root of a single plant harbours several populations of mycorrhizal and non-mycorrhizal fungi (Perotto et al., 1996a). Investigation of mycelia isolated from *Gaultheria shallon* roots (Monreal et al., 1996) has also revealed the simultaneous presence of fungi with different ITS restriction patterns.

RAPD analysis has enabled investigation on the genetic polymorphism of isolates at a higher resolution (Perotto et al., 1996a). This technique is very sensitive to reveal genetic polymorphisms among related organisms. Because of its high resolution power, the RAPD techniques has found several applications in molecular ecology and population biology (reviewed by Hadrys et al., 1992). When applied to mycological studies, it has been successfully used to identify races or even individual "clones" of filamentous fungi (Smith et al., 1992).

PCR-RAPD was used to analyse about 80 mycorrhizal mycelia isolated from *C. vulgaris* growing in five neighbouring sites, some forming conidia and identified as *Oidiodendron maius*, others growing in culture as sterile mycelia and grouped according to morphological criteria and by restriction fragments analysis. Results of PCR amplification with about ten random primers have shown a high polymorphism in *O. maius* isolates and a lower variability within populations of sterile mycelia. Many isolates of *O. maius* showing polymorphic RAPD bands were actually derived from the root apparatus of the same plant of C. *vulgaris*. These data, together with the results of

ITS/RFLP, further demonstrate that the root system of a single plant of *C. vulgaris* is a complex mosaic where several populations of mycorrhizal fungi coexist, each represented by a variable number of genetic individuals (Perotto et al., 1996a). The PCR-RAPD technique also revealed cases where similar DNA fingerprints were shared by fungal mycelia isolated from neighbouring plants of *C. vulgaris*, suggesting that networks of individual mycelia may connect the root apparatus of different host plants (Perotto et al., 1996a).

3. Occurrence of Group I Introns is a Common Feature of Ericoid Fungi

Analysis of the nuclear ribosomal genes in ericoid fungi has revealed for many isolates an unusual feature in their organization. Amplification using universal primers designed on the 18S subunits has yielded DNA fragments which were often much larger in size than expected (Fig. 1). Sequencing of these fragments has revealed that this discrepancy was due to the insertion of Group I introns. The sequence of Group I introns is characterized by four conserved regions which play a role in the formation of secondary structures (Johansen et al., 1996). These intron elements are uncommon in the nuclear ribosomal genes of eukaryotes. They occur sporadically in a few fungal species and in algae, but their role has not been elucidated. In fungi, they are abundant in the polyphyletic group of lichen-forming fungi (Gargas et al., 1995).

In ericoid fungi, one intron element inserted in the region towards the 3' end of the 18S subunit was well characterized in *H. ericae* (Egger et al., 1995). A number of additional insertions have been found for other isolates of the same species *H. ericae*, in *Oidiodendron spp.* and in most groups of ericoid sterile mycelia (S. Perotto et al., in preparation). Although their role is not known, they certainly contribute to increase genetic diversity among ericoid fungi because of their sequences and sites of insertion along the 18S rDNA. In different isolates of the same species, they can be either present or absent at specific sites (Fig. 2).

4. Functional Diversity: Production of Hydrolytic Enzymes

Ericoid fungi are capable of exploiting simple and complex organic matter commonly found in soils (Leake and Read, 1991; Leake and Miles, 1996; Cairney and Burke, 1996). In particular, *H. ericae* has been extensively investigated for its ability to grow on a variety of complex organic substrates (Leake and Read, 1991). A great proportion of the organic matter in the soil consists of the

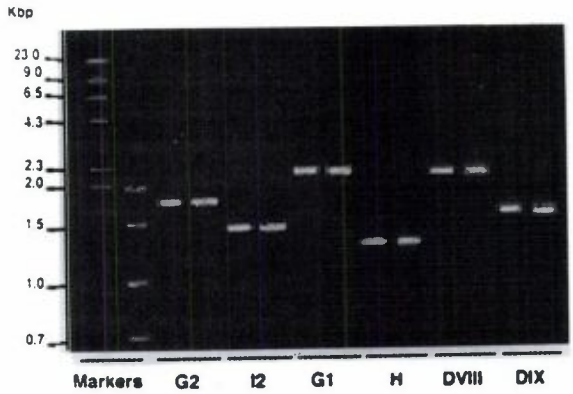

Figure 1. Separation on 1% agarose gel of PCR fragments amplified from different sterile ericoid fungal isolates using universal primers NS5 and ITS4 (White et al., 1990), which amplify about 700 bp of the 18S rDNA gene and the whole ITS region. The expected size for the fragment is about 1.3 Kbp, that was found only for sterile mycelium H. All the other isolates gave larger PCR fragments. Amplification of the sole ITS region (data not shown), gave DNA fragments of identical size for all these isolates, indicating that the insertions occur in the 18S rDNA sequence.

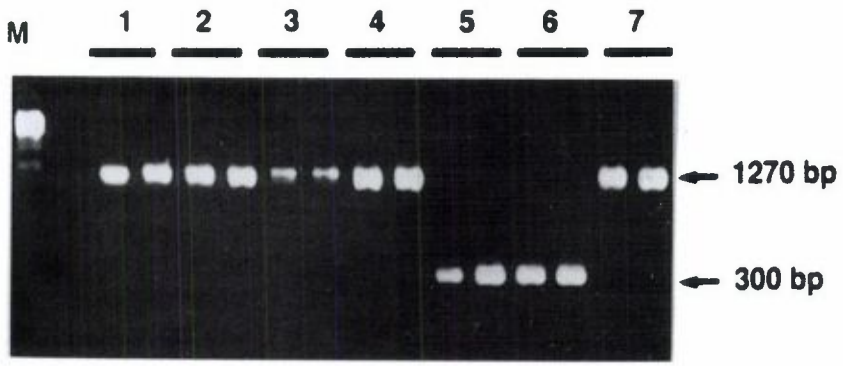

Figure 2. Ethidium bromide-stained 1.4% agarose gel showing the migration of PCR fragments amplified from different isolates of *Hymenoscyphus ericae* with universal primers NS5/NS6 (White et al., 1990). The difference in size is due to the insertion of a Group I intron. Lanes: 1) *H. ericae*; 2) *H. ericae* strain A; 3) *H. ericae* 100; 4) *H. ericae* 101; 5) *H. ericae* CV3; 6) *H. ericae* CV4; 7) *H. ericae* CV5.

polymeric components of plant and fungal cell walls, especially under conditions where microbial decomposition is slow. Ericoid fungi can utilize,

through the production and secretion of extracellular hydrolytic enzymes, polysaccharides such as pectins (Peretto et al., 1993), carboxymethylcellulose, tylose, laminarin (Varma and Bonfante, 1994) and xylans (Cairney and Burke, 1996). They can also degrade chitin, the structural polysaccharide of the fungal wall, proteins and even lignin (Leake and Read, 1991). Ericoid mycorrhizal isolates in the Myxotrichaceae and Gymnoascaceae, telomorphs of *Oidiodendron*, have also been reported to be cellulosolytic (Dalpè, 1989).

In order to investigate functional diversity of specific enzymes produced by ericoid fungi, we investigated the production of polygalacturonase (PG), an enzyme involved in the degradation of pectins, in a wide range of endophytic ericoid fungi isolated from different environments of distant geographic regions of Europe, North America and South Africa (Perotto et al., 1997). The results of biochemical analysis have shown that species belonging to the same genus (e.g. *O. citrinum*, *O. griseum* and *O. maius*) isolated from environments as different as forest soils and sandy heathlands secreted mostly acidic PG isoforms with similar mobility by solid and liquid IEF. On the other hand, PSI and PSIV, two sterile ericoid mycelia isolated in the same site but genetically distinct, were found to produce predominantly a basic and an acidic PG isoform, respectively (Perotto et al., 1997). These data suggest that the characteristics of PG isoforms may be more tightly correlated with the taxonomic position of the ericoid isolates than with the environment where these fungi grow.

Although the results discussed in Perotto et al. (1997) and Hutton et al. (1994) indicate that production of PG enzyme isoforms may be mostly related to the fungal species involved, evidences of specific functional adaptations to soil environments have derived from studies on ericoid fungi growing in polluted soils. Mycorrhizal isolates of *O. maius* have been isolated from soils either contaminated or not with heavy metals such as zinc and cadmium (K. Turnau, Krakow, Poland). Fungi isolated from polluted soils showed *in vitro*, in the presence of the same metal ions, a better growth ability when compared with isolates of the same species derived from non polluted soils (Perotto et al., 1996b; Martino et al., in preparation). When the production of polygalacturonase enzymes was analysed for these fungal strains, it was found that *O. maius* isolates derived from polluted soils produced higher amounts of PG enzyme activity, and that activity of the purified enzymes could be directly increased by zinc and cadmium, the same metal ions found in the contaminated soil. These data suggest the ability of ericoid isolates to adapt to contaminated environments, and suggest that the presence of specific PG isoforms may contribute to this adaptation.

In conclusion, recent biochemical and genetic investigations on several ericoid fungal isolates available in culture collections indicate for these fungi

quite a high degree of polymorphism, which is probably an important source of variability to allow adaptation to stressful environments. The group of ericoid fungi comprises a number of species which is likely to increase as more analyses are being carried out on genomic sequences. Preliminary results of this type of analysis, in part described in this paper, suggest that the very narrow range of specificity first described for this association is in fact wider with respect to the fungal symbionts. The same is true also for the plant hosts, since Duckett and Read (1995) have demonstrated a major extension of the host range of *H. ericae* to liverworts. Liverworts may act as a source of inoculum for ericaceous plants, because they can share the same mycobiont and often share the same environments.

We can also hypothesize that the variability observed in the biochemical characteristics of secreted enzyme isoforms, linked with the concept of multiple occupancy derived from the genetic analysis, may be of great ecological significance. Investigations on another promiscuous association, the symbiosis between corals and multiple photosynthetic microalgal endosymbionts, strongly suggest that the different physiological characteristics of the algal partners may contribute to the phenomenon of photoadaptation (Rowan and Knowlton, 1995; Rowan et al., 1997). Similarly in ericoid mycorrhiza, an individual ericaceous plant may be broadening its metabolic capabilities in the exploitation of difficult soil substrates through the association with several mycorrhizal fungi.

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