Symbiosis, 9 (1990) 167–172
Balaban Publishers, Philadelphia/Rehovot

Ericoid Fungal Strains from an Alpine Zone: Their Cytological and Cell Surface Characteristics*

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Abstract

Ericoid fungal colonies were isolated from *Calluna vulgaris* L. Hull and *Rhododendron ferrugineum* L. growing on western Alps and compared with ericoid strains previously described from northern european areas. A combination of ultrastructural, cytochemical and affinity techniques shows that the endophytes display the same features as those isolated from North Europe.

Fungal cell surface, which is deeply involved in fungus-plant adhesion and interaction, was investigated by using monoclonal antibodies. Immunological similarities with cell surface molecules present on the human pathogen *Candida albicans* suggest a common background for the adhesion processes during interaction.

Introduction

Ericoid mycorrhizae are symbiotic associations between roots of plant species belonging to Ericaceae and soil fungi mainly referred to as *Hymenoscyphus ericae* (Read) Korf and Kernan (Ascomycetes).

Even though Ericaceae display a wide geographical and ecological distribution (Meusel et al., 1978), the endomycorrhiza they form is very family-specific compared with other mycorrhizal associations.

Most of the data available on the biology of ericoid fungi (Bonfante–Fasolo and Perotto, 1988 for a complete list of references), have been obtained on strains isolated from North Europe (Duclos, 1981), with a few exception from South Africa (Straker and Mitchell, 1986) and Savoye (Duclos, 1981). Little is known about the characteristics of ericoid fungal strains isolated from plants growing in different ecological conditions.

*Reviewed

This is of particular interest since Ericaceous species colonize acidophil, peaty soil as well as calcareous, arid ones.

In the present paper some new strains were isolated from plants growing in alpine zones and the symbiotic capabilities of the new isolates were tested in an "in vitro" system. Our aims were to:

(1) characterize these new isolates by using a combination of cytological, immunological and other affinity techniques in order to compare them with the strains previously studied and already described in Bonfante–Fasolo and Perotto (1986).

(2) further analyze the ericoid fungal cell surface in order to understand those features that could be relevant to the establishment of the symbiosis.

It seems in fact that specificity in ericoid mycorrhizae comes out from a sequence of interactions between the symbionts – adhesion, penetration and compatibility – where the distribution of sugar residues at the fungal surface can be related to fungal infectivity.

Materials and Methods

Isolation of ericoid fungal strains

Fungal endophytes were isolated from roots of *Rhododendron ferrugineum* and *Calluna vulgaris* collected in a rust-red Alpenrose heath community (Carbonari valley, Piedmont, 1600 m) on western Alps following a modified method from Pearson (1971). Thin roots were carefully rinsed in water to remove soil particles and placed under a continuous stream of tap water for at least 24 hrs. The roots were then briefly surface sterilized with Na hypochloryte and crushed in a tissue grinder. Few drops from the solution were plated on 0.5% water/agar, and fungal colonies originating from plant cells were isolated and grown on 2% malt/agar medium or maintained on 2% malt extract liquid medium at 24°C. Seedlings of *C, vulgaris* L. Hull and *V. myrtillus* L. were inoculated "in vitro" with the isolates as described by Pearson and Read (1973), in order to test symbiotic capabilities.

Cytochemical and affinity techniques

Electron microscopical preparations, staining with PATAg reaction for general visualization of polysaccharides and fluorescent labelling with Concanavalin A (Con A) lectin were performed according to the protocols described in Bonfante–Fasolo et al. (1987).

Nuclear distribution was studied on fungal colonies growing on glass slides with 0.5 μg/ml DAPI solution.

Distribution of antigenic molecules was analyzed by using: (i) CB25, a monoclonal antibody raised against the ericoid fungal strain A (Pearson, 1971) of *H. ericae* and previously described in Perotto et al. (1987), (ii) GMP-1, a monoclonal antibody raised

against *Candida albicans* and recognizing a mannoprotein fraction from the fungal cell wall (Cassone et al., 1988).

Results and Discussion

All of the fungal colonies originating from root cells consist of a slow growing, dark sterile mycelium, and were able to form typical ericoid mycorrhizae "in vitro". Fungal hyphae tend to form strands (Fig. 1) in pure colture and curl in loops on the plant root surface.

Nuclear distribution was determined by using DAPI at fluorescence microscope, and hyphae always resulted to be regularly mononucleate (Fig. 2, a nucleus is arrowed). When seen at ultrastructural level, all of the new ericoid strains displayed Woronin bodies, confirming their ascomycetous nature.

An abundant extracellular material is evident on electron micrographs (Fig. 3, arrows), surrounding the cell wall of all isolates and strongly reactive to the PATAg staining. Concanavalin A-FITC on PS3 visualized mannose/glucose residues (Fig. 4) along the fungal hyphae. All of the morphological features and the lectin labelling on these isolates are perfectly comparable to those described for *H. ericae* strains (Gianinazzi–Pearson and Bonfante–Fasolo, 1986; Bonfante–Fasolo et al., 1987).

Table 1. Ericoid fungal strains*

Origin	Strains	Site
R. ferrugineum	PS1, PS2, PS3	Rifugio Barbara
C. vulgaris	PS4, PS5	Rifugio Barbara

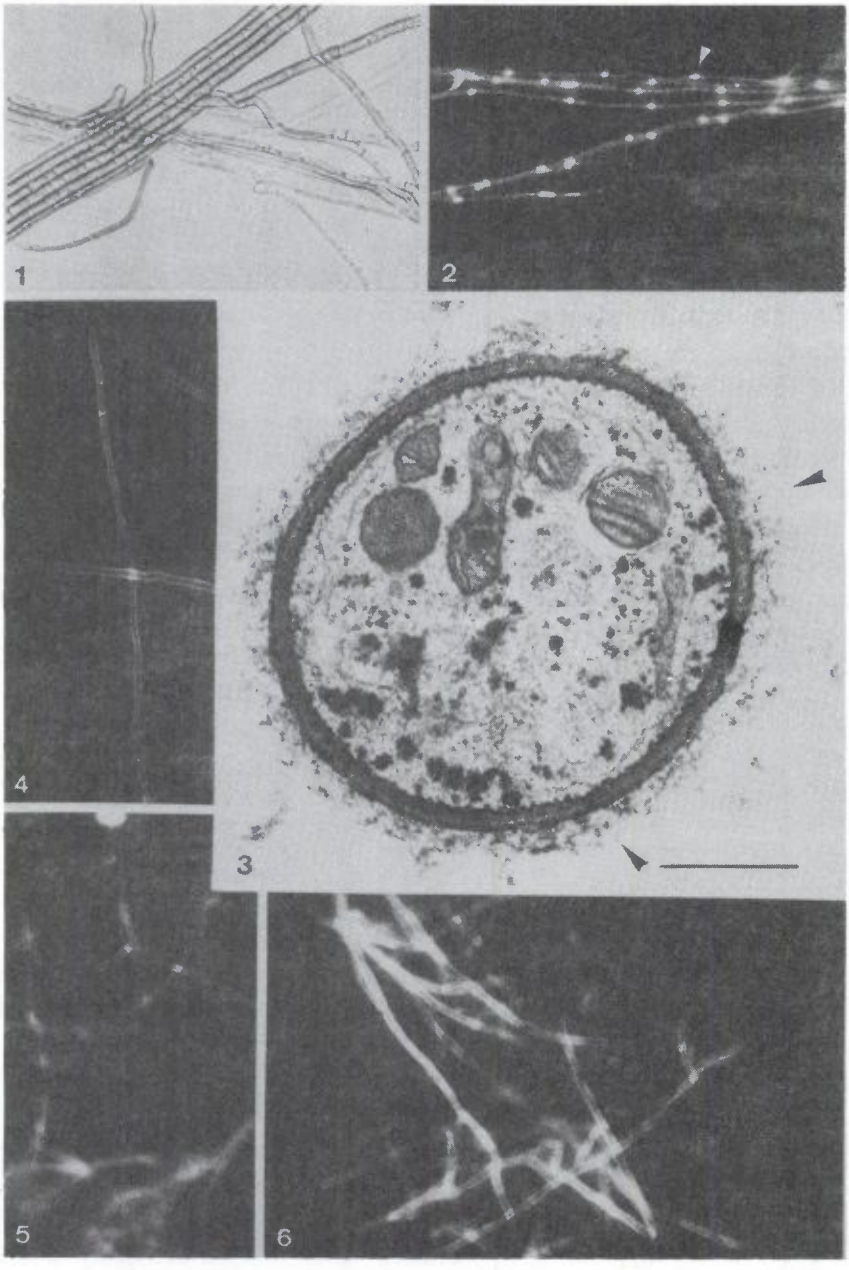
*Host plant and site of origin of ericoid fungal isolates

Observations on salt extracted fractions from intact mycelium, SDS-PAGE and ConA labelling on western blot demonstrated that part of these residues form the glycosidic chain of surface glycoproteins (Perotto et al., unpublished results).

Monoclonal antibodies raised against *H. ericae* and *C. albicans* revealed a different degree of reactivity: CB25 only weakly labelled the cell surface of ericoid fungal strain (Fig. 5), while GMP-1, the antibody raised against *C. albicans*, reacted strongly against the ericoid isolates both in pure culture (Fig. 6) and in symbiotic association.

The presence of an antigen cross-reacting with a monoclonal antibody raised against a cell surface mannoprotein isolated by *C. albicans* is extremely interesting. The fibrillar material of ericoid fungi is probably involved in the adhesion process on the epidermal cell of the host, since it disappears – or it is highly reduced – during the host colonization. Moreover, ericoid strains are not longer infective (Gianinazzi–Pearson and Bonfante–Fasolo, 1986) once they appear to have lost their capacity to produce this material.

We can suggest that the cell surface characteristics of the symbiotic fungi control



Figures. (1) Hyphae from ericoid isolates growing on glass slides tend to form strands. (2) DAPI staining on hyphae from PS3 strain shows that they are regularly mononucleate (a nucleus is arrowed). (3) An EM micrograph of strain PS3 shows the extracellular material (arrows) sheating the cell wall. (bar is 1μm). (4) ConA/FITC on hyphae of ericoid strains visualizes the presence of mannose/glucose residues. (5) Monoclonal antibody CB25 labels weakly hyphae of strain PS3. (6) Monoclonal antibody GMP-1 strongly labels hyphae of strain PS3.

the first contact with the host plants. This situation resembles the one described in the adhesion process between *C. albicans* and epithelial cells, where an extracellular fibrillar sheath is also produced and specific glycoproteins with mannose residues are required (Cassone et al., 1988).

The similarities with the *C. albicans* system is reinforced by the presence of common enzymatic activities: phosphatase activity has been demonstrated to occur in both systems (Straker et al., 1989; Tronchet et al., 1980).

We can conclude that: (1) all of the new strains isolated from an alpine zone are morphologically and biochemically indistinguishable from *H. ericae* strains isolated from other european environments, and (2) they show a molecular heterogeneity of their cell surface, but the pattern appears rather constant in the infective strains.

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