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Utilization of Cell-Wall Related Carbohydrates by Ericoid Mycorrhizal Endophytes

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Abstract

The ability of some strains of ericoid mycorrhizal fungi to utilize cell wall related mono-, di- and complex polysaccharides as sole carbon and energy source was investigated. Xylose, galactose, laminarin, tylose and carboxymethyl cellulose (CMC) supported the growth of several endophytes, such as *Hymenoscyphus ericae* (strain Read and strain A), Duclos XV, PS I, and II. By contrast, PS IV showed good growth only with laminarin and tylose. PS IV needed supplemented medium with malt or peptone extract dialysates in combination with surfactants (tween 20, tween 60 or triton-X-100) to degrade CMC. Increased growth often led to the production of loose, hyaline mycelial wefts. Commercial cellulosic materials (filter paper, scottex and microcrystalline cotton) also supported growth in *H. ericae* strain Read and PS IV as well as sterilized roots of *Calluna* and clover.

The degradation of β 1,4- and β 1,3-glucans was tested in two fungal strains (*H. ericae* strain Read and PS IV) by enzymatic assays that revealed the corresponding glucanase activities. Glucanase activities were detected when the fungi grew on CMC medium and on the sterilised root segments.

The experiments suggest the involvement of hydrolytic enzymes in the degradation of structural organic molecules present in the soil matrices and/or in the

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host cell walls: they could be produced by the ericoid mycorrhizal fungi both during the saprotrophic and symbiotic phase.

Keywords: ericoid endophytes, β 1,4-glucanase, β 1,3-glucanase, cell wall carbohydrates, cellulose

1. Introduction

In natural conditions, the fine hair roots of many Ericales are profusely colonized by some endophytes known as ericoid mycorrhizal fungi. Their role in the mineral cycling and plant nutrition is of particular importance in strongly leached and nutrient poor soils, in which nitrogen is the major growth limiting factor (Read, 1991).

During the mycorrhizal association, ericoid fungi form a loose weft around the hair roots, and hence penetrate the epidermal cells producing intracellular coils (Bonfante and Gianinazzi-Pearson, 1979). Notwithstanding this intracellular phase, they are not obligate biotrophs and therefore differ from the most common arbuscular mycorrhizal endophytes. Ericoid fungi can in fact be isolated from the host roots and maintained in culture (Pearson and Read, 1973), demonstrating saprotrophic capabilities. Investigations on their enzymatic equipment have revealed that they release extracellular enzymes such as proteases, acid phosphatases, chitinases as well as the potential to attack lignin (Leake and Read, 1990, 1991; Haselwandter et al., 1990). These enzymes may be relevant in the degradation of the structural components of the organic matrices (Read, 1991).

On the other hand, ericoid fungi overcome the barrier represented by the host cell walls in order to colonize the root cells and to establish a biotrophic relationship (Bonfante and Perotto, 1992). This suggests that the process of root colonization requires a regulated production of cell wall degrading enzymes, which allow cell invasion while maintaining host viability. Polygalacturonases produced in *in vitro* conditions by an ericoid strain have already been partially characterized (Peretto et al., 1993). Biochemical and cytochemical analyses performed on the thin hair roots of *Calluna vulgaris* have demonstrated that the root surface is structurally organized in a mucilage layer and a thick laminated cell wall. A complex network of carbohydrates is present, ranging from insoluble β 1,4-glucans to soluble sugars containing mannose, xylose, galactose and arabinose (Peretto et al., 1990).

The aim of this report was to verify the ability of some ericoid mycorrhizal endophytes to grow (1) on simple sugars, present in cell wall polysaccharides and in cell glycoproteins, and/or (2) on more complex polysaccharides such

as laminarin, tylose or carboxymethyl cellulose-Na salt as a sole carbon and energy source.

2. Materials and Methods

Ericoid fungi

Fungal isolates used in the experiments are listed in Table 1 (see Bonfante and Perotto, 1986). The cultures were maintained on plates containing 2% malt extract and 1.5% agar medium, pH 5.

Batch cultures

Strains were grown in Czapek medium in the dark at 25° C (±1) in 250 ml Erlenmayer flasks or in 50 ml incubation tubes. Each tube with 20 ml of medium was inoculated with a 5 mm disc of the test fungus cultivated on the agar medium plates, while 9 mm discs were used for the Erlenmayer flasks containing 100 ml of medium.

Czapek minimal medium contained: $NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $CaCl_2$, 0.5 g; Fe-EDTA 0.01 g and water 1000 ml; pH 5. The basal medium was modified to contain one of the following carbohydrates at a carbon concentration equivalent to 10 g/l of glucose: D-glucose; D-fructose; galactose; mannose; fucose; arabinose: D-xylose; cellobiose; sucrose; laminarin (polymer of β-1,3-glucans); tylose (polymer of β 1,4-glucans); carboxymethyl cellulose-Na-salt (CMC) and Avicel PH 101 (Fluka, Buchs, Switzerland). pH was adjusted to 5. Natural polymers employed as carbon sources were filter paper (Cartiera di Cordens, Italy), Scottex, cotton (commercial grade) and xylan oat-spelt (Fluka). In another set of experiments, root segments of *Calluna* (90 days old), clover (5 and 90 days old), and tomato (90 days old) were used. The root samples were thoroughly washed to remove adhering substratum particles, cut into 2 cm long strips, autoclaved. Each

Table 1. Ericoid fungal strains

Isolate name	Fungal species	Origin	Reference
Strain Read	*Hymenoscyphus ericae*	*Calluna vulgaris*	Read, 1974
Strain A	*Hymenoscyphus ericae*	*Calluna vulgaris*	Pearson, 1971
Duclos VIII	–	*Vaccinium corymbosum*	Duclos and Fortin, 1983
Duclos IX	–	*Erica vagans*	Duclos and Fortin, 1983
Duclos XV	–	*Vaccinium corymbosum*	Duclos and Fortin, 1983
PS I	–	*Rhododendron* sp.	Perotto et al., 1990
PS II	–	*Rhododendron* sp.	Perotto et al., 1990
PS IV	–	*Calluna vulgaris*	Perotto et al., 1990

incubation tube contained one of the natural carbon substrates at a rate of 0.2 g/20 ml medium.

Where indicated, nutrient media were modified to include the dialysates of complex organic nutrients (see below) and surfactants. Tweens used were tween 20 (polyoxyethylene and sorbitanmonolaurate) or tween 60 (polyoxyethylene sorbitanmonostearate) (Sigma, St. Louis, USA) and triton-X-100 (polyethylene glycol tert-octylphenyl ether) (Fluka). They were added at a concentration of 0.25% (w/v).

Dialysate from complex organic nutrients

Two grams each of malt extract (Sigma, St. Louis, USA) and of bacteriological peptone (Oxoid, England) were individually suspended in 20 ml distilled water. The suspension was dialysed (Visking dialysis tubing, exclusion size 12–15 kDa, Serva, GmbH, Germany) for 24 hr against 90 ml of 50 mM sodium acetate buffer, pH 5 at 4° C. Buffer containing the extruded low molecular weight polypeptides, sugar materials and trace elements (Rajaram and Varma, 1990) were autoclaved and stored at 4° C, henceforth termed nutrient dialysates.

Biomass

The growth of the fungi was determined as the dry weight biomass of the mycelium. After 4–6 weeks, the mycelia were harvested by vacuum filtration, dried in an oven at 60° C for 48 hr and then weighed. Three replications were made for each treatment.

Enzyme assays

Culture samples were passed through filter paper placed on Büchner funnel to remove the fungal mycelium. Culture media were centrifuged at 10,000 g for 10 min and the supernatant was dialysed (Visking dialysis tubing, exclusion size 12–15 kDa) for 16 hr against 50 mM Na-acetate buffer (pH 5), and 0.15 M NaCl. Sample concentration was performed by employing vacuum rotatory evaporator (Büchi Rotavapor-R) at 30° C. Samples were stored at −20° C. β-1, 4-glucanase (CMCase) (E.C.3.2.1.4.) and β-1,3-glucanase (E.C.3.2.1.39.) assays were performed according to Mullings (1985) and Paul and Varma (1990). The reaction mixture contained 1% (w/v) CMC (Sigma), or 0.5% (w/v) laminarin (from *Laminaria digitata*, Fluka), 50 mM Na-acetate buffer, pH 5, and dialysed crude extract (10–100 μl) in a total volume of 300 μl in Eppendorf tube. The assay was run for 90 min at 30° C. To analyse the

substrate specificity of the glucanases, microcrystalline cellulose Avicel, particle size 50 μm was used instead of CMC. The formation of reducing sugars was determined photometrically at 540 nm (Varian DMS 100 Spectrophotometer) according to Somogyi (1952).

One unit of enzyme activity was defined as the amount of enzyme producing 1 micro equivalent of reducing groups min^{-1} (RGU).

3. Results

Fungal growth on defined media

Galactose, xylose, laminarin and tylose supported the growth of *H. ericae* (Read strain) as well as sodium salt of CMC (Table 2), while in contrast, PS IV showed good growth only with laminarin and tylose. Both strains showed poor to intermediate ability to utilize glucose, fructose, mannose, fucose, arabinose and sucrose as well as cellobiose (Table 2).

The optimal concentrations for galactose, xylose and CMC ranged from 1.5% to 2% (w/v) (Figs. 1 & 2). These carbon sources also promoted the growth in some of the other ericoid fungi (strain A, Duclos VIII, XV, PS I and II). Duclos IX failed to grow in all the different conditions tested (Table 3). None of the ericoid strains utilised Avicel and xylan (data not shown). In the

Table 2. Biomass and hyphal characteristics of two ericoid strains growing on various carbohydrates used as carbon sources in Czapek minimal medium. Each value (mg) represents the mean of three replications

	Fungal strains			
	H. ericae Strain Read		PS IV	
Carbon sources	Biomass (mg)	Hyphal characteristics	Biomass (mg)	Hyphal characteristics
glucose	1.4	tc	2.9	tc,c
fructose	2.7	tc	2.4	tc,c
galactose	22.1	tw,h	4.1	tc,c
mannose	11.0	tc,c	4.5	tc,c
fucose	5.6	tc,c	2.7	tc,c
arabinose	3.7	tc,c	1.9	tc,c
xylose	18.8	tc,c	3.9	tc,c
cellobiose	14.9	tc,c	6.3	tc,c
sucrose	9.7	tc,c	3.8	tc,c
laminarin	21.4	lw,h	15.7	lw,h
tylose	19.9	tw,h	18.7	lw,h
CMC	22.9	tc,c	5.0	tc,c

Little growth was detected when no carbon source was included (values not shown). Legends: tc, thickly interwoven compact; lw, loose weft; h, hyaline; c, grey black; CMC, carboxymethyl cellulose.

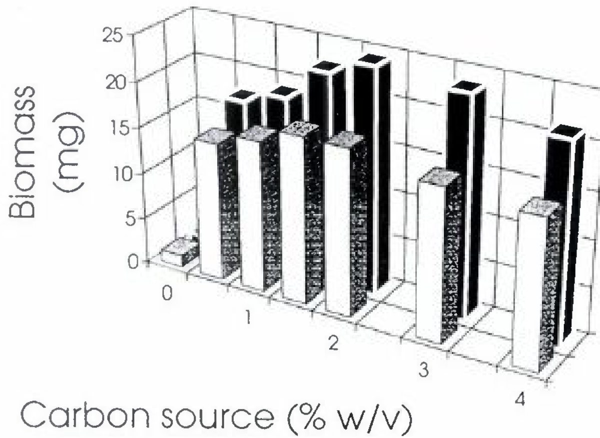

Figure 1. Growth of *Hymenoscyphus ericae* (strain Read) on galactose (white columns) and xylose (black columns). Control media did not contain sugars.

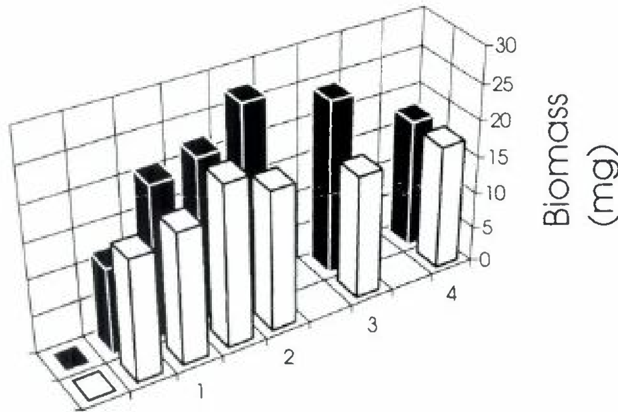

Figure 2. Growth of *H. ericae* strain Read (white columns) and PS IV (black columns) on Czapek minimal medium with carboxymethyl cellulose (CMC). Salt concentrations were 0.5, 1.0, 1.5, 2.0, 3.0 4.0 (w/v). No addition served as control. The incubation medium for PS IV was supplemented by malt extract dialysates (10% v/v) and tween-20 (0.25% w/v). Total volume was standardized to 20 ml/tube.

Table 3. Biomass and hyphal characteristics of ericoid fungi growing on various carbohydrates used as carbon source in Czapek minimal liquid medium. Each value (mg) represents the mean of three replications.

Strains	Carbon source Biomass (mg) with hyphal characteristics							
	Galactose		Xylose		Tylose		CMC	
Strain A	7.4	tc	16.9	lw,h	11.3	lw,h	15.3	tc,c
Duclos VIII	2.0	tc	3.7	tc	5.4	tc	16.1	tc,c
Duclos IX	1.8	tc	1.4	tc	3.7	tc	4.9	tc
Duclos XV	3.1	tc	19.7	lw,h	14.9	lw,h	13.9	tc
PS I	2.9	tc	20.1	lw,h	10.8	lw,h	18.8	lw,h
PS II	10.5	tc	15.8	lw,h	9.7	lw,h	2.9	tc

Little growth was detected when no carbon source was not included (values not shown).
Legends: tc, thickly interwoven compact; lw, loose weft; h, hyaline; c, grey black.

growth-promoting media, ericoid mycorrhizal fungi produced a loose and hyaline mycelial weft (Tables 2 & 3), instead of the compact, gray-black colonies, often formed on agar media (Pearson, 1971). In the further experiments only results dealing with *H. ericae* (Read strain) and PS IV are reported to illustrate the growth pattern.

Minimal medium supplemented with the nutrient dialysates in combination with surfactants such as tween-20 or 60 and triton-X-100 strongly supported the growth on CMC in the strain PS IV (Table 4). However, these positive effects were not found on *H. ericae*. Both the fungi showed only negligible growth when incubated on Czapek minimal medium alone or supplemented with dialysate nutrients without CMC. None of the additives individually could promote growth.

Natural polymers as growth substratum

H. ericae (Read strain) and PS IV profusely grew on the sterilised root segments of *Calluna* and on 5 day old clover. In contrast, fungal growth on root segments from 90 days old clover was relatively less and tomato root segments failed to sustain any growth. Cellulosic substrates like scottex and filter paper proved to be good carbon sources as evident by the profuse fungal mycelium production. By contrast, laboratory grade microcrystalline cotton served as a relatively poor substratum. In all these experiments, the fungal growth was not quantitatively estimated, due to the difficulties of separating the fungal mass from the substrates, while the culture filtrates were used for the enzyme assays.

Table 4. Effect of nutrient dialysates and surfactants on fungal biomass (mg, 35 d) and production of endoglucanase (RGU $\times 10^{-3} \times$ ml^{-1} culture filtrate, 28 d) in two strains of ericoid fungi. The value for enzyme activity represents the mean of three analyses of each replication.

Medium	*H. ericae* Strain Read		PS IV	
	Biomass	β-1,4 glucanase	Biomass	β-1,4 glucanase
CMC	111.0	14.2	8.1	0.5
CMC + tw-20	90.4	11.7	8.9	0.2
CMC + tw-60	97.4	9.9	10.4	0.3
CMC + trit-X-100	5.4	0.4	6.4	0.2
CMC + MeD	98.6	9.6	7.5	0.4
CMC + MeD + tw-20	103.3	12.8	215.0	46.6
CMC + MeD + tw-60	102.7	9.9	201.6	22.1
CMC + MeD + trit-X-100	3.8	8.5	185.3	5.2
CMC + PeptD	81.8	8.4	9.3	0.4
CMC + PeptD + tw-20	94.5	8.6	174.7	23.2
CMC + PeptD + tw-60	98.3	5.8	189.9	9.5
CMC + PeptD + trit-X-100	4.2	4.9	86.7	3.7

Legends: tw, tweens; trit, triton (0.25% w/v); MeD and PeptD, malt and peptone extracts dialysate (10% v/v), respectively.

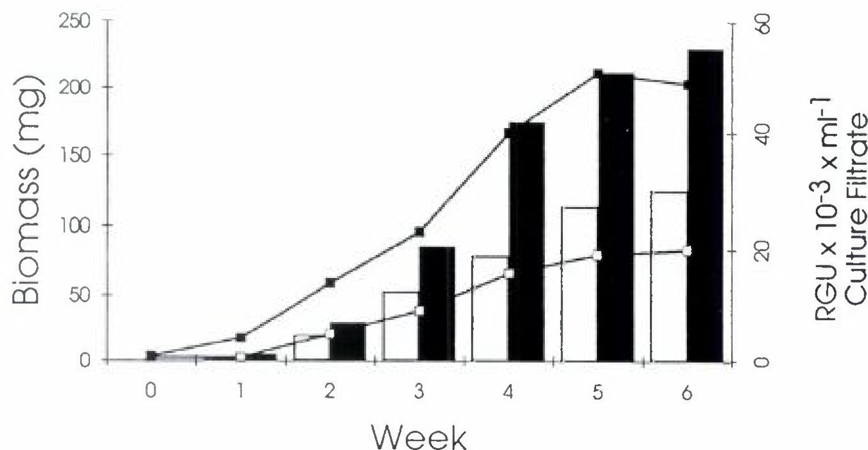

Figure 3. Time course of CMC (1% w/v) consumption and synthesis of the corresponding enzymes by *H. ericae*, strain Read (white columns and line) and PS IV (black columns and line). CMCase analysis was performed in triplicates for each replication. Values are the average of nine tests. Nutrient extracts dialysate (malt/peptone) and the surfactants (tweens/triton-X-100) individually or in combinations did not interfere with enzyme assays.

Table 5. Glucanase activities (RGU $\times 10^{-3} \times$ ml^{-1} culture filtrate) of two strains of ericoid fungi grown with sterilized root segments. Experiments were conducted in duplicates, incubated at 25° C (±1) for 42 d in dark. The value represents the mean of three analyses of each replication.

Root segments	*Hymenoscyphus ericae*		PS IV	
	β-1,4 glucanase	β-1,3 glucanase	β-1,4 glucanase	β-1,3 glucanase
Calluna vulgaris (90 day old)	11.0	23.0	12.9	14.0
clover (5 day old)	15.5	17.0	9.1	12.0
clover (90 day old)	4.8	15.3	6.3	6.3
tomato (90 day old)	nd	nd	nd	nd

nd, not detected

Glucanases activities

β 1,4-glucanase (CMCase) activity was evaluated by reducing-end groups assay, in crude dialysed extracts. When fungi were grown on CMC as the sole carbon source, a measurable amount of CMCase was detected within a week of incubation. However, very low activity was detected in PS IV as compared to the *H. ericae* strain (Table 4). Supplementing the medium with nutrient dialysates and tweens 20 and 60, cellulase activity was promoted in PS IV (Table 4). None of these additives individually could promote the enzyme activity. Addition of glucose up to 50 mM to a medium containing CMC did not inhibit the cellulase activity (data not shown). A correlation between enzyme activity and fungal growth was found until 28–35 days from inoculation (Fig. 3). Similar to the growth, the optimal enzyme production was obtained at 1.5–2% CMC in the incubation minimal medium.

β-1,4-glucanase and β-1,3-glucanase activities were detected in the culture filtrates when the fungal strains were grown in the presence of root materials form *Calluna* and clover (Table 5). Poor to moderate amounts of cellulase were also assayed in the incubation minimal medium from cotton, scottex, and filter paper, respectively (data not shown).

4. Discussion

The eight strains of mycorrhizal ericoid fungi tested in the cultivation experiments demonstrated the ability to utilise cell wall related sugars as sole carbon source, producing measurable quantities of biomass. Xylose, an important component of hemicelluloses, and galactose, present in pectic polysaccharides as galactans and in cell wall glycoproteins (Brett and Waldron, 1990), supported a good fungal growth. These results confirm previous studies reporting the nutritional requirements of some ericoid fungi for their *in vitro* growth

(Pearson, 1971; Harley and Smith, 1983). In addition, the experiments demonstrate that suspended "gelly-like" and soluble polymers of β 1,4-glucans (tylose and carboxymethyl cellulose-Na salt, respectively) and of β 1,3-glucans (laminarin) are good substrates for the fungal growth. By contrast, native and crystalline cellulosic materials as well as xylans were not degraded.

Ericales, which are the natural host plants for ericoid endophytes, are characterized by epidermal cells, whose outer wall is particularly thickened and rich in cellulose organized in helicoidal layers (Berta and Bonfante, 1990; Peretto et al., 1990). The two strains, which have been studied in detail in these experiments (*H. ericae* and PS IV), degrade not only pectins and polygalacturonic acids (Peretto et al., 1993), but also cellulose-related polymers thanks to the production of cellulases. This capacity raises some questions on the molecular mechanisms underlying the penetration process *in planta*. Due to the presence of the substrate (cellulose in the epidermal cell wall) and of its enzyme (cellulase), it could be suggested that cellulases are involved in the process of the establishment of the mycorrhizal symbiosis. However, experiments on cellulases of plant origin have shown that these enzymes are more active in hydrolysing soluble cellulose and hemicelluloses like xyloglucans, while, despite their name, have little impact on native cellulose (Fry, 1993). These results lead to the hypothesis that ericoid fungi, as the majority of biotrophs (Heiler et al., 1993), may produce *in planta* only low levels of cellulase. They might degrade cellulose in a well regulated way, for example, only at the sites of their penetration points of the host plants. By contrast, they could be good glucan-degrading organisms during their saprotrophic phase.

Laminarin, consisting of β 1,3-glucans, represented a good substrate for both strains. The enzyme assay demonstrates that the ericoid fungi produce the corresponding enzyme, thanks to which growth is allowed. Interestingly, β 1,3-glucans are present in the wall of the endophytes, as skeletal components together with chitin both *in vitro* and during the symbiotic phase (unpublished results). These findings suggest that β 1,3-glucanase may be important in the construction of the fungal wall, which is usually considered the result of a balance between synthetic and lytic activities (Fevre et al., 1991). On the other hand, β 1,3-glucans are usual components in plant cell walls as callose and together with β 1-4-glucans form unbranched molecules, which are important components of hemicelluloses in the walls of some plants (Brett and Waldron, 1990). These findings suggest that β-1,3 glucanase could be active together with the β-1,4 glucanase in the hemicellulose degradation (i.e.: xyloglucans and β-1,4- and β-1,3-glucans). However, so far no information is available on the hemicellulose composition of Ericales.

Notwithstanding the fact that *H. ericae* and PS IV show good symbiotic

capabilities and that both colonize heather roots in a comparable way (Bonfante et al., 1987, Perotto et al., 1990), they showed some differences in their biochemical characteristics, for example CMC degradation by the strain PS IV only occurred in the presence of nutrient dialysates plus surfactants. The latter are known to facilitate the secretion of hydrolytic enzymes across the plasma membrane and the periplasmic space in several bacteria (Aubert et al., 1988; Gilkes et al., 1991; Varma et al., 1993). It could be suggested that surfactants may be operative also in some fungi in a similar way and that they cooperate with the low molecular weight polypeptides present in the dialysates to give a stimulatory effect.

The physiological differences between the two ericoid fungi would not be surprising, since they do not seem to be taxonomically related (S. Perotto, unpublished results): on the basis of its DNA fingerprinting PS IV differs both from *H. ericae* group and from the ericoid symbionts related to the genus *Oidiodendron* (Dalpe', 1986). Molecular probes have already been used to distinguish ericoid mycorrhizal fungi: the amplification of portions from the ribosomal DNA have demonstrated a relatedness between *Scytalidium vaccinii*, a hyphomycete, and *H. ericae*, as well as a divergence with *H. monotropae* (Egger and Sigler, 1993).

In conclusion, this work has shown that polymers of β 1,4- and β 1,3-glucans are used by ericoid mycorrhizal fungi as sole carbon sources. When grown on these substrates, they produce and secrete the corresponding cell wall degrading enzymes as revealed by enzyme assays. This suggests the involvement of such enzymes (1) during the saprotrophic phase in the degradation of complex organic polymers present in the soil matrices, and (2) during the symbiotic phase in the degradation of polysaccharides which are structural components of the host cell wall.

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REFERENCES

Aubert, J.P., Beguin, P., and Millet, J. 1988. *Biochemistry and Genetics of Cellulose Degradation.* Academic Press, New York.

Berta, G. and Bonfante, P. 1990. Anatomy and cytology of hair roots in *Calluna vulgaris. G. Bot. Iral.*. **123:** 9–23.

Bonfante, P. and Gianinazzi-Pearson, V. 1979. Ultrastructural aspects of endomycorrhiza in the Ericaceae. I. Naturally infected hair roots of *Calluna vulgaris* L. Hull. *New Phytol.* **83:** 739–744.

Bonfante, P. and Perotto, S. 1986. Visualization of surface sugar residues in mycorrhizal ericoid fungi by fluorescein conjugated lectins. *Symbiosis* **1:** 269–286.

Bonfante, P. and Perotto, S. 1992. Plants and endomycorrhizal fungi: The cellular and molecular basis of their interactions. In: *Molecular Signals in Plant-Microbe Communication.* Ed. by D.P.S. Verma. CRC Press, Boca Raton, FL, pp. 445–467.

Bonfante, P., Perotto, S., Testa, B., and Faccio, A. 1987. Ultrastructural localization of cell surface sugar residues in ericoid mycorrhizal fungi by gold-labelled lectins. *Protoplasma* **139:** 25–35.

Brett, C. and Waldron, K. 1990. *Physiology and Biochemistry of Plant Cell Walls.* Unwin Hyman, London.

Duclos, J.L. and Fortin, J.A. 1983. Effect of glucose and active charcoal on *in vitro* synthesis of ericoid mycorrhiza with *Vaccinium* spp. *New Phytol.* **94:** 95–102.

Dalpe', Y. 1986. Axenic synthesis of ericoid mycorrhiza in *Vaccinium angustifolium* Ait. by *Oidiodendron* species. *New Phytol.* **103:** 391–396.

Egger, K.N. and Sigler, L. 1993. Relatedness of the ericoid endophytes *Scytalidium vaccinii* and *Hymenoscyphus ericae* inferred from analysis of ribosomal DNA. *Mycologia* **85:** 219–230.

Fevre, M., Girard, V., and Nodet, P. 1991. Cellulose and β-glucan synthesis in *Saprolegnia.* In: *Tip Growth in Plant and Fungal Cell.* I.B. Heath, ed. Academic Press, New York, pp. 97–107.

Fry, S.C. 1993. Loosening the ties. *Current Biology* **3:** 355–357.

Gilkes, N.R., Henrisst, D.G., Kilburn, D.G., Miller, R.C., and Warren, R.A.J. 1991. Domains in microbial β-1,4-glycanases: Sequence conservation, function, and enzyme families. *Microbial Reviews* **55:** 303–312.

Harley, J.L. and Smith, S.E. 1983. *Mycorrhizal Symbiosis.* Academic Press, New York.

Haselwandter, K., Bobleter, and Read, D.J. 1990. Degradation of ^{14}C-labelled lignin and dehydropolymer of coniferyl alcohol by ericoid and ectomycorrhizal fungi. *Arch. Mikrobiol.* **153:** 352–354.

Heiler, S., Mendgen, K., and Deising, H. 1993. Cellulolytic enzymes of the obligately biotrophic rust fungus *Uromyces viciae-fabae* are regulated differentiation-specifically. *Mycol. Res.* **97:** 77–85.

Leake, J.R. and Read, D.J. 1990. Chitin as a source for mycorrhizal fungi. *Mycol. Res.* **94:** 993–995.

Leake, J.R. and Read, D.J. 1991. Experiments with Ericoid mycorrhiza. In: *Experiments with Mycorrhizae*, Vol. 23. J.R. Norris, D.J. Read and A.K. Varma, eds. Academic Press, New York, pp. 435–457.

Mullings, R. 1985. Measurements of saccharification by cellulase. *Enzymes & Microb. Technol.* **7**: 586–591.

Paul, J. and Varma, A. 1990. Influence of sugars on endoglucanase and β-xylanase activities of a *Bacillus* strain. *Biotechn. Lett.* **12**: 61–64.

Pearson, V. 1971. The biology of the mycorrhizae in the ericaceae. Ph.D. thesis, University of Sheffield, UK.

Pearson, V. and Read, D.J. 1973. The biology of mycorrhizae in the Ericaceae. I. The isolation of the endophyte and synthesis of the mycorrhizas in aseptic culture. *New Phytol.* **72**: 371–379.

Perotto, S., Peretto, R., Moré, D., and Bonfante, P. 1990. Ericoid fungal strains from an alpine zone: their cytological and cell surface characteristics. *Symbiosis* **9**: 167–172.

Peretto, R., Bettini, V., and Bonfante, P. 1993. Evidence of two polygalacturonase isoforms produced by a mycorrhizal ericoid fungus during its saprophytic growth. *FEMS Microbiol. Lett.* **114**: 85–92.

Peretto, R., Perotto, S., Faccio, A., and Bonfante, P. 1990. Cell surface in *Calluna vulgaris* L. hair roots. *Protoplasma* **155**: 1–18.

Rajaram, S. and Varma, A. 1990. Production and characterization of xylanases from *Bacillus thermoalkalophilus* growth on agricultural wastes. *J. Appl. Microbiol. Biotechnol.* **34**: 141–144.

Read, D.J. 1974. *Pezizella ericaceae* sp. nov. the perfect state of a typical mycorrhizal endophyte of ericaceae. *Trans. Br. Mycol. Soc.* **63**: 381–383.

Read, D.J. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–390.

Somogyi, N. 1952. Notes on sugar determination. *J. Biol. Chem.* **195**: 19–23.

Varma, A., Balakrishna, K., Paul, J., Saxena, S., and Konig, H. 1993. Lignocellulose degradation by microorganisms from termite hills and termite guts. *FEMS Microbiol. Rev.* (in press).