Utilization of major detrital substrates by dark-septate, root endophytes[1]

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Abstract: Utilization of major forms of carbon, nitrogen and phosphorus commonly present in plant litter and detritus was determined for cultures of *Phialophora finlandia*, *Phialocephala fortinii* and five dark-septate, root endophyte isolates from alpine plant communities. All cultures utilized cellulose, laminarin, starch and xylan as sole carbon source. Protein and ribonucleic acids were hydrolyzed by all cultures as sole nitrogen and phosphorus sources, respectively. The fatty acid ester, Tween 40, was hydrolyzed by all cultures. None of the cultures decolorized two polymeric dyes used as presumptive tests for lignolytic activity. These hydrolytic capabilities suggest that these dark-septate root endophytes, either as biotrophs or saprotrophs, are able to access major organic detrital nutrient pools.

Key Words: decomposition, enzyme production, *Mycelium radicis atrovirens*

Phialocephala fortinii and *Phialophora finlandia* have been identified as putatively mycorrhizal members of the heterogeneous complex of dark-septate, root endophytes (DSE) partially or completely overlapping with a heterogeneous morphotype of sterile, root-inhabiting fungi, *Mycelium radicis atrovirens* (MRA) (Wang and Wilcox 1985, Stoyke and Currah 1991, Stoyke et al 1992, Jumpponen et al 1998). Jumpponen and Trappe (1998) reviewed reports of DSE associated with nearly 600 plant species globally distributed. Although their mycorrhizal status is uncertain (Jumpponen et al 1998), DSE are commonly associated with many alpine, boreal and northern temperate herb, shrub and tree species (Haselwandter and Read 1980, Ahlich and Sieber 1996, Jumpponen and

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Trappe 1998). In such environments, Read (1991) has suggested biotrophic fungi would be adapted to accessing organic nutrient pools. This implies the ability to produce the necessary extracellular enzymes to breakdown the complex detrital macromolecules to assimilable subunits.

Whether biotrophic or saprotrophic, the ability of DSE to access litter and detrital carbon, nitrogen and phosphorus pools is poorly known. Bååth and Söderström (1980) found an MRA isolate capable of hydrolyzing protein, but not chitin. Mullen (1995) demonstrated utilization of organic nitrogen by DSE isolates from *Ranunculus adoneus*. Because of the potential importance of DSE in the nutrition of host plants, we report here on the ability of DSE fungi to breakdown the major polymeric forms of carbon, nitrogen and phosphorus commonly found in plant detritus and soil organic matter.

Cultures of *Phialophora finlandia* and *Phialocephala fortinii*, along with six sporulating and sterile isolates identified morphologically and by ITS-RFLP (Jumponnen and Trappe 1996) as *P. fortinii* (TABLE I) were maintained on 1/3 strength potato dextrose agar (Difco). Hydrolytic capacities were determined on a basal medium (Caldwell et al 1991) supplemented with the appropriate target substrate (below). Triplicate plates were incubated for 2–4 wk at 22 C. Inoculated basal media plates without test substrate and uninoculated reaction plates were run as controls.

Polysaccharide hydrolysis was determined with 1% starch ($\alpha[1,4]$ glucan), carboxymethylcellulose (CMC, $\beta[1,4]$ glucan) and laminarin ($\beta[1,3]$ glucan) as the sole carbon source. Positive activity was determined by color reaction after staining with iodine solution for starch or congo red for CMC, laminarin or xylan (Teather and Wood 1982). Hydrolysis of a fatty acid ester, Tween 40, was determined by formation of an opaque halo of calcium palmitate crystals in basal medium supplemented with 1% Tween 40 (Caldwell et al 1991). Protein hydrolysis was determined by formation of a clear halo in basal medium with gelatin (4 g/L) as the sole nitrogen source (Gerhardt 1981). Ribonucleic acid (RNA) hydrolysis was determined by chromatic shift (blue to pink) of toluidine blue O (200 mg/L, Gerhardt 1981) in basal medium with RNA (2 g/L) as sole phosphorus source. Presumptive lignolytic activity was determined by decoloration of azure B (Archibald 1992) and remazol-brilliant blue (RBB, Ulmer et al 1984) added to nitrogen-limited (1 mM) basal medium.

The type culture (FAP-7) and six field isolates of *P. fortinii*, along with *P. finlandia*, all hydrolyzed the polysaccharide substrates: starch, cellulose, laminarin

TABLE I. Type cultures and dark-septate root endophyte field isolates screened for substrate reactions

Fungi	Host plant	Reference/Collector
Phialophora finlandia (UAMH 8151)	*Pinus strobus*	Kronick-Ursic
Phialocephala fortinii (FAP7)	*P. sylvestris*	Wang and Wilcox 1985
Field isolates		
SE24[a]	*Lupinus latifolius*	O'Dell et al 1993
EC-01[b]	*Salix* sp.	Cazares and Trappe
EC-11[c]	*Phyllodoce glanduliflora*	Cazares and Trappe
1-3a1[b]	*P. empetriformis*	Jumpponen and Trappe 1996
2-1b1[b]	*Juncus drummondii*	Jumpponen and Trappe 1996
2-3 salix I[b]	*S. communata*	Jumpponen and Trappe 1996

[a] Conidia and conidiophores produced.

[b] Monomorphic to *P. fortinii* type by ITS-RFLP (Jumponnen and Trappe 1996).

[c] Macromorphologically similar to *P. fortinii*.

and xylan (TABLE II). Cellulose and xylans are major structural polysaccharides in plants, starch is a major storage polymer, and β[1,3] glucans are common in fungal cell walls (Gooday and Trinci 1980) and soil polysaccharides (Cheshire 1979). Other MRA isolates have previously been found to hydrolyze cellulose, pectin and xylan (Flanagan and Scarborough 1974, Richard and Fortin 1974, Bååth and Söderström 1980, Fernando and Currah 1996), but not starch (Flanagan and Scarborough 1974). Hydrolysis of these polymers could provide both a labile substrate and breakdown cell wall barriers to access nutrient-rich cytoplasmic residues.

TABLE II. Substrate reactions common to dark-septate root endophytes type cultures and field isolates

	Phialocephala fortinii			
	FAP7	SE24	DSE isolates[a]	*Phialophora finlandia*
Carbon				
Polysaccharide				
Cellulose	+	+	5[b]	+
Laminarin	+	+	5	+
Starch	+	+	5	+
Xylan	+	+	5	+
Fatty Acid Ester	+	+	5	+
Polymeric Dyes[c]				
Azure B	−	−	0	−
RBB	−	−	0	−
Nitrogen				
Protein	+	+	5	+
Phosphorus				
Ribonucleic Acid	+	+	5	+

[a] Similar to *P. fortinii* type either macromorphologically or by ITS-RFLP (TABLE I).

[b] Number of substrate-positive isolates out of five tested.

[c] Presumptive lignolytic substrates (Archibald 1992, Ulmer et al 1984).

All our DSE cultures and isolates hydrolyzed the synthetic fatty acid ester, Tween 40 (TABLE II). This activity has not been previously reported in this group of fungi, although Caldwell et al (1991) suggested this trait a characteristic of ectomycorrhizal fungi in environments with accumulations of organic matter. Hydrolysis of major environmental fatty acid esters, lipids and waxes, could provide energy and carbon, as well as breach cuticular wax barriers to allow access to nutrient-rich cytoplasmic residues.

Both gelatin and RNA were hydrolyzed by all our DSE cultures and isolates. The gelatin hydrolysis results conform to a previous report of proteolytic activity by an MRA isolate (Bååth and Söderström 1980), while this is apparently the first report of nucleic acid hydrolysis by DSE. The ability of biotrophic DSE to hydrolyze protein-N and nucleic acid-P would provide a mechanism for the host plant to access these major detrital N and P pools from the accumulating litter in the pioneer plant communties at glacier forefront.

We found no evidence for lignolytic enzymes using complex polymeric dyes (TABLE II), although phenoloxidase production by DSE has been reported (Flanagan and Scarborough 1974, Currah and Tsuneda 1993, Fernando and Currah 1995). This supports the previous findings of Currah and Tsuneda (1993) that phenoloxidase production by *P. fortinii* was unrelated to lignin degradation.

Our results demonstrate that DSE fungi are clearly capable of producing the extracellular enzymes necessary to process major detrital C, N and P polymers into usable subunits. In a biotrophic context, these activities by DSE would also allow the host plant access to N and P in those environments where nutrients accumulate in organic pools.

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