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FATTY ACID ESTERASE PRODUCTION BY ECTOMYCORRHIZAL FUNGI

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Several reports have indicated the stimulating effect of fatty acid-containing materials on the growth of ectomycorrhizal fungi. Growth of different ectomycorrhizal fungi can be stimulated by lipids (Schisler and Volkoff, **1977**; Fries *et al.*, **1985**), Tween 80 (Straatsma and Bruinsma, **1986**), or certain free fatty acids (Lindeberg and Lindeberg, **1974**). Palmer and Hacskaylo (**1970**) reported no growth stimulation using the synthetic lipid triacetin, but this may have been the result of acetate inhibition (Lindeberg and Lindeberg, **1974**) or the inability to use acetate as a sole carbon source.

Fatty acid esters are cleaved by enzymes, including lipases (*sensu stricto* triacylglycerol acylhydrolase, E.C. 3.1.1.3), which can release free fatty acids from several sources, including lipids, phospholipids, sterol esters, waxes, cutin, and suberin. Although lipolytic activity, frequently using Tween substrates, has been studied in various saprophytic fungi from diverse environments (Das *et al.*, **1979**; Egger, **1986**; Gessner, **1980**; Gochenaur, **1984**; Zare-Maivan and Shearer, 1988) and food materials (e.g., Roberts *et al.*, **1987**), few ectomycorrhizal fungi have been examined.

Fungal strains (TABLE I) were obtained from the USDA Forest Service, Pacific Northwest Research Station, Corvallis, Oregon, RWU-4503 cuture collection, except for *Hebeloma crustuliniforme* which was provided by the USDA Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, Oregon. All strains were maintained on an ectomycorrhizal basal medium (Pearson and Read, **1975**) modified to reduce phosphate levels (Giltrap and Lewis, **1981**) and increase Ca^{2+} concentration (Sierra, **1957**). The medium consisted of 10 g glucose, 0.25 g ammonium tartrate, 0.4 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 50 mg yeast extract, 5 mg ferric citrate, 5 mg $MnSO_4 \cdot 4H_2O$, 4 mg $ZnSO_4 \cdot 7H_2O$, and 15 g agar (per liter distilled water). To detect fatty acid esterase production, a supplement of 1% (v/v) of either Tween 20, 40, or 80 was added. The medium was adjusted to pH 5.5 and autoclaved (121 C, 15 min). Plates were inoculated in duplicate or triplicate with halves of a 5 mm plug cut from the margin of an actively growing culture. Control plates of fungi grown on medium without Tween added were used to evaluate possible excretion of oxalic acid that might cause a false-positive reaction. Plates were incubated at room temperature for up to 60 days and periodically examined for growth and production of the characteristic halo resulting from the precipitation of fatty acid-calcium salts (Sierra, **1957**).

Of 48 strains tested, 25 produced characteristic calcium-fatty acid salt halos on one or more of the three Tween substrates tested (TABLE I), with 17 detected on Tween 20, 25 on Tween 40, and 18 on Tween 80. In several cases, Tween inhib-

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TABLE I

REACTION ON TWEEN ESTERS BY ECTOMYCORRHIZAL FUNGI

Taxon	Strain	Habitat[a]	Reaction on Tweens[b] 20	40	80
Alpova olivaceotinctus (Smith) Trappe	8245		(−)	(−)	−
Arcangeliella parva Thiers	9467	M	(+)	(+)	+
Austrogautieria sp.	7873	M	(−)	(+)	(+)
Byssoporia terrestris	Z 30	W	+	+	+
Cenococcum geophilum Fr.	A145A		−	−	−
Chondrogaster sp.	9833	M	+	+	+
Cortinarius magnivelatus (Morse) Thiers & Smith	9537	M	+	+	−
Elaphomyces sp.	8646		(−)	−	−
Endogone (immature)	S566		(−)	(−)	(−)
Gautieria caudata (Harkn.) Zeller & Dodge	8481	M	+	+	+
G. cf. *graveolens*	8724	M	+	+	+
G. monticola Harkness	AG-7	M	+	+	+
Genabea cerebriformis (Halen.) Trappe	6264	W	(+)	+	+
Hebeloma crustuliniforme (Bull. ex. St. Am.) Quel.	HECR2		(−)	−	−
Hydnangium carneum Wallroth	9831		(−)	−	−
Hymenogaster albus Berk. & Br.	9832		(−)	(+)	(−)
Hysterangium coriaceum Hesse	AH-7	M	+	+	+
H. crassirhachis Zeller & Dodge	AH-6	M	+	+	+
H. gardneri Fischer	8405	M	+	+	+
H. inflatum Fischer	9787	M	(−)	+	+
Hysterangium sp.	4154	M	+	+	+
Laccaria bicolor (Maire) Pat.	S238		(−)	−	−
Leccinum scabrum (Bull. ex. Fr.) S. F. Gray	8967		(−)	(+)	(+)
Leucophelps spinispora Fogel	7435		(−)	(−)	(−)
Melanogaster variegatus (Vittadini) Tul. & Tul.	8222		(−)	(−)	(−)
Mycoamaranthus auriobis Castellano, Trappe & Malajczuk	4045	M	+	+	−
Paxillus involutus (Butsch: Fr.) Fr.	8969		(−)	−	−
Piloderma croceum Erikks. & Hjortst	163	M	+	+	+
Pisolithus tinctorius (Pers.) Coker & Couch	S359		−	−	−
Radiigera atrogleba Zeller	9470	W	+	+	+
Rhizopogon clavitisporus Smith	S412		(−)	(−)	−
R. colossus Smith	S548		(−)	(−)	−
R. ellenae Smith	8974		−	−	−
R. evadens Smith	8972		(−)	−	−
R. hawkerae Smith	A104		(−)	(−)	−
R. occidentlais Zeller & Dodge	7544		−	−	−
R. ochraceisporus Smith	9009		(−)	−	−
R. ochraceorubens Smith	7555		(−)	−	−
R. reaii Smith	8073		−	−	−
R. vinicolor Smith	7534		−	−	−
R. vulgaris (Vitt.) M. Lange	A56		−	−	−
Sarcodon scabrosus (Fr.) Bourd. & Galz.	8727		(−)	−	−
Tricholoma magnivelare (Peck) Redhead	7088		−	+	−
Tuber aestivum Vittadini	491		(−)	+	−
T. borchi Vittadini	S211A		(−)	+	(−)
T. uncinatum Chatin	S491		−	+	−

[a] Habitat: M—forms mat structures; W—associated with decomposing wood.

[b] Reaction: (−) no growth, no esterase reaction; (+) no growth, but esterase positive reaction; − growth with no esterase reaction; + growth with positive esterase reaction.

ited growth from the fungus inoculum plug onto the agar medium, although absence of growth did not always correspond to lack of fatty acid esterase (e.g., *Arcangeliella parva*). Growth suppression and lower frequency of esterase detection using Tween 20 was probably caused by the increased toxicity of either the C_{12} fatty acid or its Tween ester (Kabara, **1987**). This suggests that caution be exercised when using this substrate alone in screening fatty acid esterase production

(e.g., Hankin and Anagnostakis, **1975**). Oxalate production (precipitation of calcium oxalate crystals) was not detected on any of the control plates.

Most positive reactions seemed limited to a distinct group of presumptive ectomycorrhizal fungi that form extensive hyphal or rhizomorph mat structures in forest soils. Of particular interest were the results for those ectomycorrhizal fungi, *Gautieria monticola, Hysterangium coriaceum,* and *H. crassirhachis,* that produce these mat structures in the upper soil layers of Douglas-fir forests in the Pacific Northwest. The mats formed by these fungi and *H. setchellii,* which was not available for inclusion in this survey, are loci of significantly altered microbial activities and soil chemistry (Cromack *et al.*, **1979**, **1988**; Griffiths *et al.*, **1987**, **1990**; Sollins *et al.*, **1981**). *Hysterangium gardneri* forms mats in the litter layer of *Eucalyptus globulus* plantations in northern California, and *H. inflatum* forms mats in association with *Eucalyptus* in Australia. Isolates of other mat-forming ectomycorrhizal fungi strongly positive for hydrolysis of the Tween additives included *G. caudata, G. othii, Mycoamaranthus auriobis, Piloderma croceum,* and unidentifiable species of *Austrogautieria, Chondrogaster,* and *Hysterangium.* Three ectomycorrhizal fungi frequently with decomposing wood, *Byssoporia terrestris, Genabea cerebriformis* and *Radiigera atrogleba,* also hydrolyzed all three Tween additives.

Some isolates, not associated with mat formation or decomposing wood, produced limited reactions in the presence of Tween additives. These included taxa that are considered late successional fungi (Dighton and Mason, **1985**), e.g., *Leccinum scabrum* and *Tricholoma* species. Dighton and Mason (**1985**) have suggested such fungi have higher energy demands than early successional fungi (e.g., *Hebeloma crustuliniforme*) and are able to use organic nutrient pools.

The capacity of ectomycorrhizal fungi to hydrolyze fatty acid esters has several potential benefits. Assimilated fatty acids could be taken up by the fungus and incorporated into new fungal lipids or cleaved into acetate subunits by β-oxidation and used either for energy or biosynthesis. Energy supplementation, in addition to that normally provided by the host tree, could be particularly important for those ectomycorrhizal fungi that form extensive mat structures with peripheral mycelia distant from the root-fungus interface or for late successional fungi with high energy requirements (Dighton and Mason, **1985**). Alternatively, the acetate subunits or acetyl CoA could be directed through different biosynthetic pathways (Garroway and Evans, **1984**) to produce a wide variety of cellular materials as well as secondary metabolites.

Fatty acid esterases may also influence the mineral nutrition of those mat-forming ectomycorrhizal fungi in forests where accumulating detritus ties up increasing quantities of nitrogen and phosphorus in organic pools. While colonizing fresh litter, the initial capacity to penetrate or disrupt wax or suberin barriers would allow hyphal or enzymatic access to otherwise occluded pools of organic nitrogen and phosphorus.

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