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Production and characterization of exocellular proteases in ectomycorrhizal fungi

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Introduction

Mycorrhizas grow very often in soil layers in which organic nitrogen compounds are present in large quantities. The ability of several ectomycorrhizal fungi to assimilate proteins and to transfer their nitrogen to plants has already been demonstrated (Abuzinadah and Read, 1986; Abuzinadah *et al.,* 1986). These results suggest that proteases are excreted by the fungi and protease activities detected in mycorrhiza-forming fungi were reported by Lyr (1963) and Ramstedt and Söderhall (1983). In this study, the process of production of exocellular proteases has been investigated in free living fungi as well as in mycorrhizas. Experiments were carried out with *Amanita rubescens* and *Lactarius subdulcis* which live in organic horizons and with *Cenococcum geophilum* and *Hebeloma crustuliniforme* which live predominantly in mineral soil layers.

Materials and Methods

The fungi were collected from mycorrhizas. They were grown in shaken cultures at 25°C in Pachlewski's medium containing nitrogen as either diammonium tartrate or proteins: bovine serum albumin (BSA), gelatin, casein or proteins extracted from the litter (Botton *et al.,* 1986). Enzyme activities were determined in culture filtrates, either by spectrophotometry or by spectrofluorometry using fluorescein isothiocyanate-labeled BSA (FITC–BSA) as described by Twining (1984). Proteins purified from the litter by ammonium sulfate fractionation and DEAE–cellulose chromatography were also labeled with FITC and used as a substrate and as inducers.

Results

Protease activity remained at a very low level when the fungus was cultivated in the presence of ammonium, but increased strongly after transfer of the organism to fresh medium containing casein, gelatin or BSA as the sole nitrogen source. Gelatin proved to be the most efficient inducer (Fig. 1a). Elimination of carbon, inorganic nitrogen or sulfur was not sufficient to induce exocellular proteases, proteins also had to be present. However, in nitrogen- and carbon-deficient media, gelatin was not efficient (not shown). A group of proteins of about 46 kDa was purified from the forest litter and was a better inducer than gelatin, in spite of its lower concentration, both in *C. geophilum* and *H. crustuliniforme* (Fig. 1b).

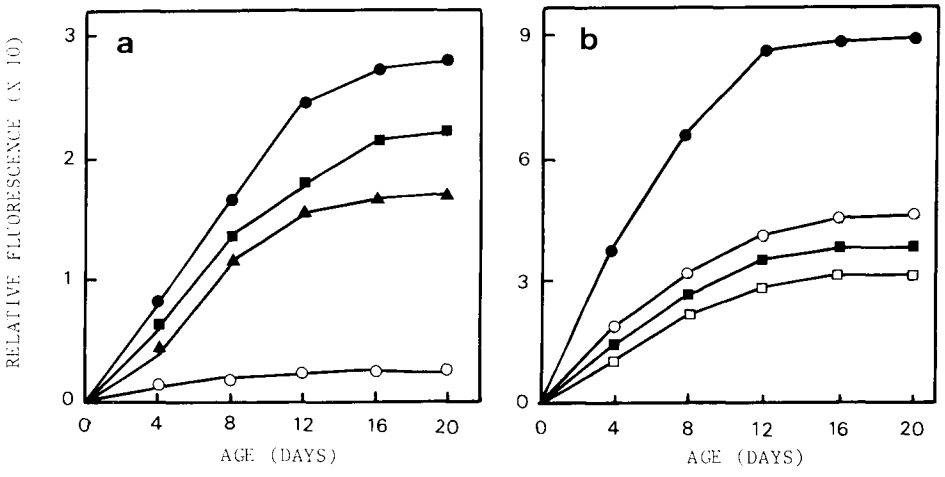

Fig. 1. Induction of protease activities in ectomycorrhizal fungi. **a.** After transfer of *C. geophilum* to medium containing casein (▲), gelatin (●) or BSA (■). The control was transferred onto ammonia-containing medium (○) **b.** *C. geophilum* induced by 0.2% gelatin (❑) and by 0.1% proteins extracted from the litter (■). *H. crustuliniforme* induced by 0.2% gelatin (○) and by 0.1% proteins extracted from the litter (●).

As shown in Table I, *A. rubescens* and *L. subdulcis* secreted larger amounts of exocellular proteases than *C. geophilum* and *H. crustuliniforme*, although growth of the 2 former fungi was considerably reduced. For the 4 fungi tested, controls with ammonium (Pachlewski's medium) were better developed than the cultures grown in the presence of proteins from the litter, but protease excretion was reduced.

Protease activity was also induced in beech–*Lactarius* ectomycorrhizas incubated in the presence of litter proteins. Gelatin was less efficient and ammonium repressed the excretion after 48 h of incubation. Non-mycorrhizal roots did not exhibit protease activity (Fig. 2).

Table I. Protease induction and growth of 4 ectomycorrhizal fungi cultivated in the presence of 0.1% proteins from the litter (induced) or in the presence of ammonium (Pachlewski's medium).

	Dry mass (mg)	*Exocellular protease activity / thallus (relative fluorescence)*
C. geophilum		
induced	20.5	44
control	38.9	3.2
H. crustuliniforme		
induced	18	30
control	34.6	2.9
A. rubescens		
induced	11.3	198
control	18.9	6.9
L. subdulcis		
induced	8.6	180
control	15.4	5.3

Conclusion

These results indicate that ectomycorrhizal fungi secrete significant amounts of proteases when the culture medium is supplemented with proteins. Very often, alkaline proteases were detected in the medium; however, when the fungi were

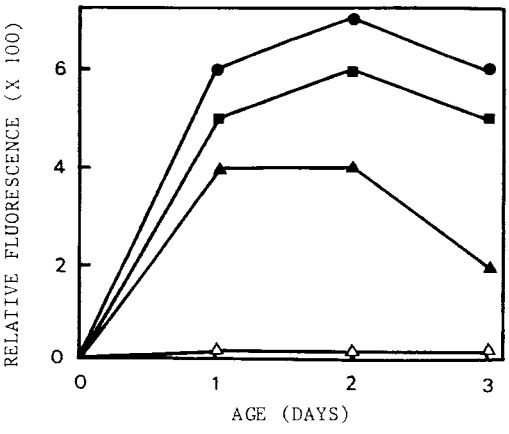

Fig. 2. Protease activity of beech–*Lactarius* ectomycorrhizas. Mycorrhizas incubated in Pachlewski's medium (▲), in NH_4^+-deficient medium supplemented with gelatin (■), in NH_4^+-deficient medium supplemented with proteins purified from the litter (●); non-mycorrhizal roots incubated in NH_4^+-deficient medium supplemented with proteins purified from the litter (△). Proteins from the litter were used as a substrate to determine protease activities.

induced with proteins from the litter and proteases were assayed with the same substrate, optimum pH of activity was about 5. It is likely that the proteases excreted enable the organism to use extracellular proteins as a source of nitrogen and a close correlation exists between the ability of the fungi to produce such enzymes and their distribution in the soil layers.

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