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Pectic Enzymes Produced by Fungal Root Associates of Alpine Plants

By

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With 7 Figures

Summary

Haselwandter K. 1983. Pectic enzymes produced by fungal root associates of alpine plants. — Phyton (Austria) 23 (1): 55—64 with 7 figures. — English with German summary.

Roots of many alpine plants are infected with dark septate fungal hyphae, but little is known of the physiology, function and ecological significance of these associations. The production and activity of pectic enzymes has been determined in a number of isolates of this type of fungus. Such enzymes facilitate penetration of host cell walls. It has been shown that all of the isolates synthesize pectic enzymes though the level of activity of these enzymes and their pH-optima varied between different strains. It is concluded that the isolates have the capacity readily to penetrate plant cells enzymatically and that the nature of the resulting host — fungus relationship is determined by subsequent post-penetration events.

Zusammenfassung

Haselwandter K. 1983. Die Bildung pektinolytischer Enzyme durch mit Wurzeln alpiner Pflanzen assoziierte Pilze. — Phyton (Austria) 23 (1): 55—64, mit 7 Abbildungen. — Englisch mit deutscher Zusammenfassung.

Die Wurzeln vieler alpiner Pflanzen sind mit dunklen, septierten Pilzhyphen infiziert. Über die Physiologie, Funktion und ökologische Bedeutung dieser Assoziation ist aber nur wenig bekannt. Eine Reihe von Isolaten dieses

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Pilztyps wurde auf die Bildung und Aktivität pektinolytischer Enzyme unter-
sucht. Solche Enzyme erleichtern das Durchdringen der Zellwände der Wirts-
pflanze. Es konnte gezeigt werden, daß alle Isolate pektinolytische Enzyme
synthetisieren, wobei allerdings das Ausmaß der Aktivität dieser Enzyme und
ihr pH-Optimum zwischen den verschiedenen Isolaten variiert. Es wird
daraus geschlossen, daß die Isolate die Fähigkeit besitzen, pflanzliche Zellen
enzymatisch schnell zu durchdringen, und daß die Art der sich daraus ergebenden
Beziehung zwischen Wirtspflanze und Pilz durch spätere Ereignisse nach dem
Eindringen bestimmt wird.

Introduction

Roots of plants in alpine grass heath as well as in the nival zone are
frequently infected by fungi with dark septate (DS) hyphae (HASELWANDTER
& READ 1980). Some of these fungi resemble those previously described as
Rhizoctonia (PEYRONEL 1924, SAKSENA & VAARTAJA 1960) while others
are similar to *Phialophora* (CAIN 1952, DEACON 1973). This type of infection
occurs either in addition to that formed by vesicular-arbuscular mycorrhizal
fungi or, as in the case of *Carex* species, it may represent the dominant
infection type (READ & HASELWANDTER 1981). Marked growth stimulation
and enhancement of tissue phosphorus content has been observed when
DS fungal isolates from *Carex* roots are re-inoculated onto aseptically grown
Carex seedlings (HASELWANDTER & READ 1982).

In view of the fact that the relationship is of a mutualistic nature it is
of interest to examine the basis of the infection process. This paper describes
an analysis of the possible role of enzymes in host penetration. Despite
some controversy in the literature (DRYSDALE & LANGCAKE 1973) it
appears that enzymatic activity plays a primary role in plant cell-wall
penetration and mechanical factors are of secondary importance (WHEELER
1975). Pectic enzymes seem to be the key factor in cell-wall degradation
(BATEMAN & MILLAR, 1966, BATEMAN & BASHAM 1976). In the infection
process plant cell walls must first be attacked by such an enzyme (endo-
polygalacturonase) prior to further degradation by other enzymes, such as
arabinosidase, cellulase and pronase (ENGLISH et al. 1972, JONES et al. 1972).
In order to assess the capability of fungal root associates to produce pectic
enzymes several different fungal strains were grown in a nutrient medium
containing pectin, and the culture filtrate was subsequently assayed for
pectinolytic activity.

Materials and Methods

Fungi with DS hyphae were isolated from a range of important alpine
plants as described elsewhere (HASELWANDTER & READ 1982). Since all
isolates produced only vegetative mycelia it has not been possible to provide
a precise identification. The strains chosen for analysis were obtained from
Carex (C3, C4), *Festuca* (F3), *Nigritella* (N1), and, for comparative purposes,

three taxonomically established *Rhizoctonia* species. C3, C4 and F3 are among the most important root associates of their host species in alpine plant communities. N1 is a typical orchid endosymbiont.

Cultures of *R. anomala, R. alpina,* and *R. endophytica* var. *endophytica* were obtained from Centraalbureau voor Schimmelcultures, Baarn, Netherlands. *R. anomala* was originally isolated from *Orchis maculata,* and *R. alpina* from *Viola palustris. R. endophytica* var. *endophytica* was isolated by SAKSENA & VAARTAJA (1960) from healthy *Pinus banksiana* seedlings, and their inoculation experiments suggested that this species is an endophyte of various conifers and angiosperms.

The experimental approach was similar to that described by PÉROM-BELON & HADLEY (1965). The fungal strains were first grown for 25 days at 18° C on a medium containing 1% glucose, 0.35% casein hydrolysate, 0.05% yeast extract, 0.1% KH_2PO_4 and 0.05% $MgSO_4.7H_2O$, pH 5.5. The mycelia were transferred to 20 ml Ringer solution (Merck No. 10113) and blended for 10—15 seconds to form a suspension. Aliquots of the suspension were dispensed in bulk at a ratio of 1 : 60 into the experimental medium which was of the same composition as described above but with 1% pectin (Sigma No. P-9135) substituted for glucose. Quantities of 30 ml of the inoculated experimental medium were dispensed with continuous stirring into 100 ml — Erlenmeyer flasks. Cultures were maintained at 18° C and harvested (3 replicates per harvest) at appropriate intervals over a period of 22 days. Culture media were filtered, their pH was determined, and the filtrates were stored at —20° C prior to enzyme analyses.

Enzyme assays

The activity of polygalacturonase (PG, E. C. 3.2.1.15, = pectinase) and pectinesterase (PE, E. C. 3.1.1.11, = pectinmethylesterase) was determined viscometrically using a Brookfield LVT Micro-Viscometer. Nomenclature of pectic enzymes follows BYRDE & WILLETTS (1977). The reaction mixture consisted of 2 ml culture filtrate, 3 ml McIlvaine buffer (McILVAINE 1921) and 5 ml 1% sodium polypectate (Sigma No. P-1879) or pectin (Sigma No. P-9135), both adjusted to the desired pH. The reaction time was 1 hr, and the reaction temperature 25° C. The results, expressed as % loss in viscosity, were calculated for the reaction mixtures containing either active or heat-inactivated (15 min/100° C) filtrate. Loss in viscosity is considered to represent a sensitive means of estimating activity of pectic enzymes (BATEMAN 1963). Since the optimum pH for any single pectic enzyme variies with the enzyme source, and is species and even strain specific, optima for PG and PE activities were determined for each isolate over the range pH 4.0 to 7.0 at 0.5 unit intervals.

Results and Discussion

All fungal isolates showed measurable levels of pectic enzyme activity *in vitro*. However, the rate of production and activity varied with different strains. The strains C3, C4, F3, *R. alpina* and *R. endophytica* var. *endophytica* exhibited the greatest levels of enzyme activity (Fig. 1—5). The orchid endophytes N1 and *R. anomala* produced lower pectic enzyme activity and the differences between these isolates were small (Fig. 6—7).

Since the reaction time was short and exo-PG and exo-PE reduce viscosity only slowly (PÉROMBELON & HADLEY 1965) it is most likely that the observed loss in viscosity of the reaction mixture is attributable to endo-PG and endo-PE. The results, however, may be influenced by the presence of additional pectic enzymes. Pectin lyase, for example, also degrades polygalacturonic acid (LUND & MAPSON 1970; BYRDE & WILLETTS 1977), though it is most active under alkaline conditions (HALL & WOOD 1970, ROMBOUTS & PILNIK 1972).

The pH-optimum of PG varied between the isolates in the range 4.0 to 6.0, and that of PE between 4.5 and 6.5 (Table 1). PÉROMBELON & HADLEY (1965) also found that the pH-optima of different pectinolytic enzymes varied with different strains. It is possible that the pH-optimum of PG of isolates F3, *R. anomala* and *R. alpina* were somewhat lower than pH 4.0 but because of the risk of chemical breakdown of the enzymes
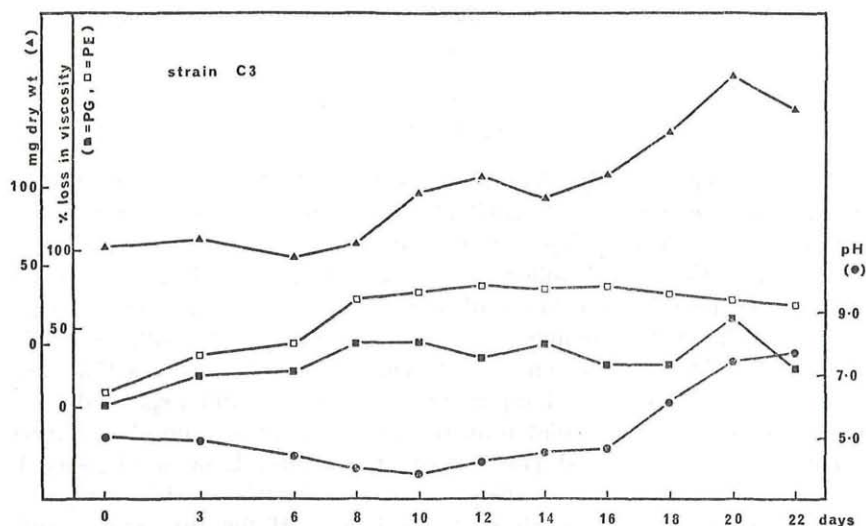

Fig. 1. Strain C3: The activity of the pectic enzymes polygalacturonase (PG, — ■ —) and pectinesterase (PE, — □ —), the obtained dry weight (— ▲ —) and pH (— ● —) of medium in culture

under both alkaline and extremely acid conditions (DEUEL & STUTZ, 1958) assays below pH 4.0 were not attempted.

All of the isolates showed higher activity against pectin than sodium polypectate. This is in contrast to observations of some workers (TALBOYS & BUSH 1970, BISEN & AGARWAL 1980) with other fungi. In addition in
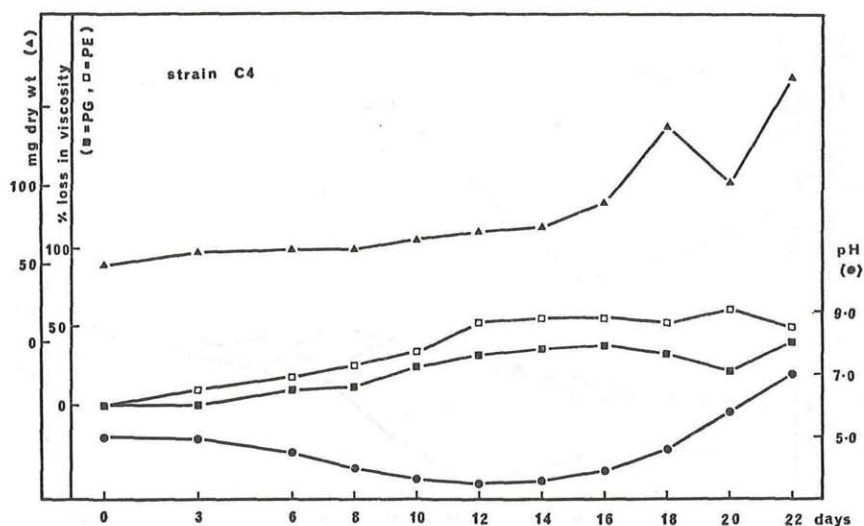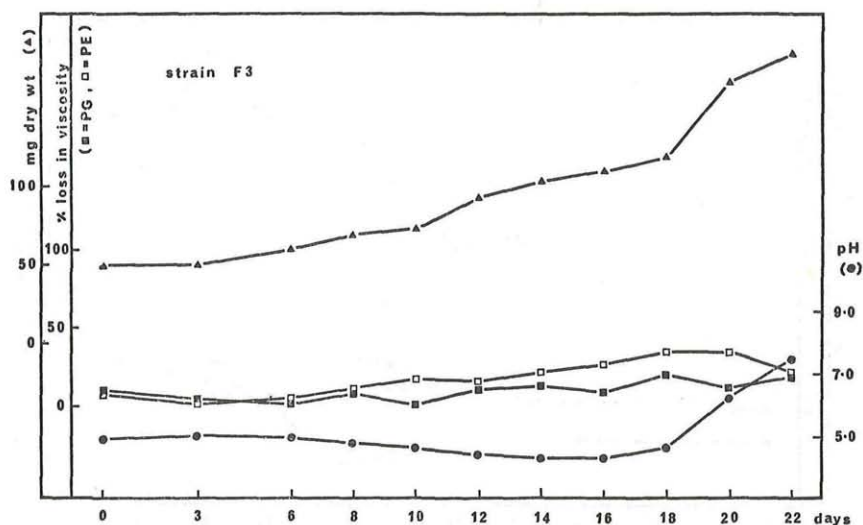

Fig. 2. Strain C4. Symbols see Fig. 1

Fig. 3. Strain F3. Symbols see Fig. 1

the present experiment pre-cultivation on a pectin rich medium may have stimulated production of PE relative to PG.

There is little correlation between production fo pectic enzymes and total amount of fungal growth in culture (Fig. 1—7). A rapid increase in
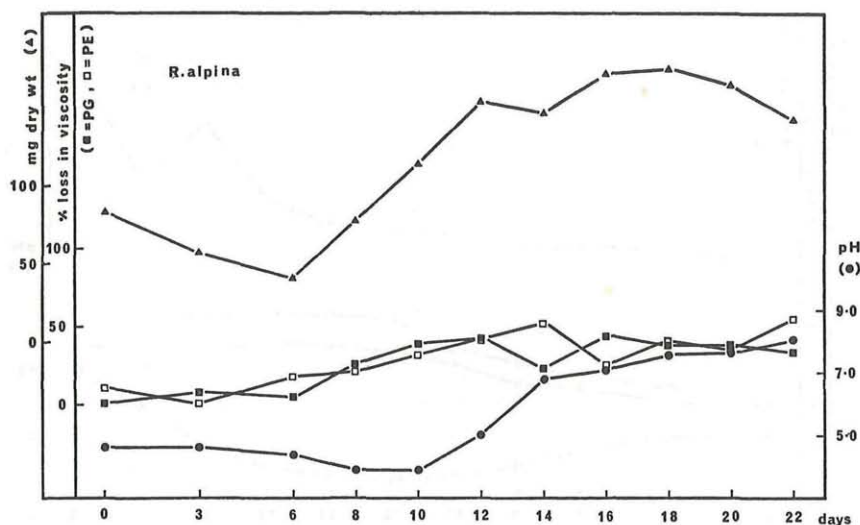

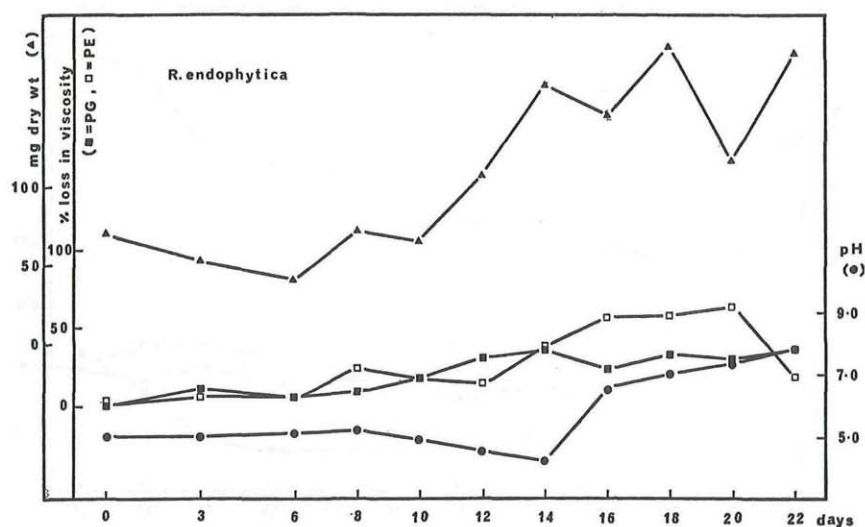
Fig. 4. *Rhizoctonia alpina.* Symbols see Fig. 1

Fig. 5. *Rhizoctonia endophytica.* Symbols see Fig. 1

mycelial growth in most strains occurs after about 10 days and a rise in pH of the culture medium follows the increase in growth rate.

While cell wall penetration will be facilitated by pectic enzymes it is likely that the requirement for enzyme activity varies with stage of the infection process. This may explain why pectic enzyme production is an
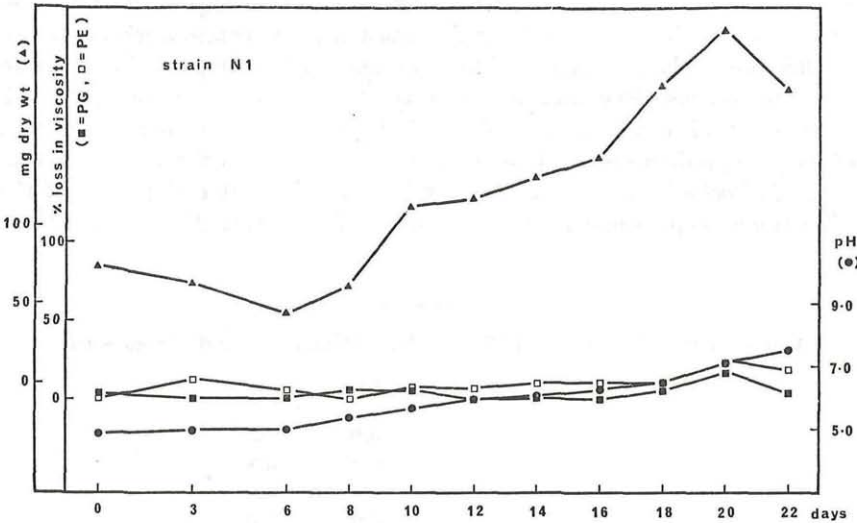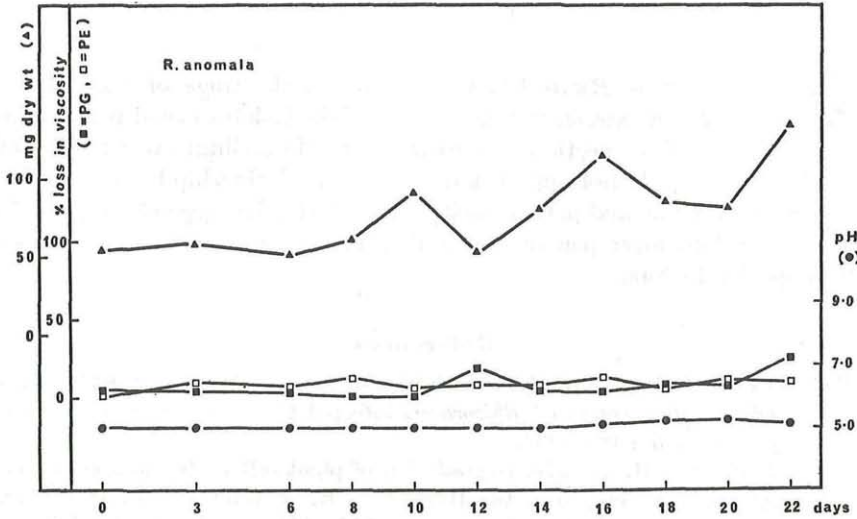

Fig. 6. Strain N1. Symbols see Fig. 1

Fig. 7. *Rhizoctonia anomala*. Symbols see Fig. 1

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inducible phenomenon (PÉROMBELON & HADLEY 1965). It is likely that they are formed only in an early phase of infection and that they are subsequently inactivated, as suggested by DEVERALL & WOOD (1961).

The primary role of pectic enzymes in plant pathogenic infection processes has been stressed by Van der PLANK (1975) who stated that "cell walls must be attacked by endopolygalacturonase before they can be further degraded by other enzymes". Since endo-symbiotic fungi must also penetrate cell walls in order to establish a mutualistic association with an autotrophic host plant a similar infection mechanism may be involved in these organisms. PÉROMBELON & HADLEY (1965) confirmed that such mechanisms also exist in symbiotic orchid endophytes and that the pattern of enzyme production was indistinguishable from that exhibited by pathogenic *Rhizoctonia* strains. It has now been shown that endophytes of the *Rhizoctonia* type some at least of which form mutualistic associations

Table 1

Approximate pH-optima of PG and PE activity of the different strains

Strain	PG	PE
C3	6.0	4.5
C4	6.0	5.5
F3	4.0	4.5
N1	5.0	6.5
R. anomala	4.0	6.5
R. alpina	4.0	6.5
R. endophytica	5.5	5.5

(HASELWANDTER & READ 1982) occur in a wide range of host plants (READ & HASELWANDTER 1981). Since all of the isolates tested possess the capacity to produce pectic enzymes which would facilitate penetration of the host it is clearly not appropriate to stress a relationship between pectic enzyme production and pathogenicity *sensu strictu*. The type of relationship which develops after penetration is determined by other, fungus and/or host specific factors.

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