Neth. J. Pl. Path. 98 (1992) 77-80 SHORT COMMUNICATION

Glycolytic activities in some fungicolous fungi

T. HIJWEGEN

Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, the Netherlands

Accepted 25 November 1991

Abstract

Some fungal species isolated from other fungi were tested for their capacity to degrade chitin, cellulose and laminarin. Only three entomophagous fungi degraded native chitin. However colloidal chitin was degraded by all fungi tested except four. Cellulolytic activity was mainly found in fungi with a poor performance against cucumber powdery mildew. This suggests that the capacity to produce cellulase is not important for successful mycoparasitic activity against cucumber powdery mildew. A good correlation was found between the capacity to degrade laminarin and the mycoparasitic activity of the fungi that were investigated.

Additional keywords: biological control, hyperparasites, cell wall degradation, enzymes

In a survey of the mycoparasitic activities of 17 fungicolous fungi against cucumber powdery mildew, *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll., several of them appeared to have potential as biocontrol agents (Hijwegen, 1988).

It is generally accepted that mycoparasites exert their influence on host fungi either by producing toxins or enzymes or both. The effects of culture filtrates of these 17 fungi against cucumber powdery mildew have been reported (Hijwegen, 1989). However, no correlation could be found between the antifungal activities of culture filtrates, assumed to contain mainly toxins, and the effects of spraying spores on the pathogen. This prompted us to investigate the possibility of cell wall-degrading enzymes being involved in mycoparasitism. Therefore the chitinolytic, cellulolytic and glucanolytic capacities of these fungi were investigated in in vitro systems containing chitin, cellulose or laminarin.

The fungi (listed in Table 1) were obtained as described previously (Hijwegen, 1988). The fungus *Verticillium psalliotae* had been incorrectly identified as *V. fungicola* in our previous reports and on the basis of recently published evidence *Tilletiopsis albescens* of earlier publications should now be named *T. pallescens* (Boekhout, 1991).

For the determination of cellulolytic activities, the fungi were precultured on cellulose-agar. One cm^2 strips from the colony margin were added to 5 ml of an autoclaved suspension of 1% cellulose-azure (Sigma) in 0.2 M phosphate buffer pH 4.7 and incubated at 22 °C for 8 days (modified after Thompstone & Dix, 1985). The mixture was then filtered and the extinction of the resulting filtrate measured colorimetrically at 595 nm. The experiment was repeated three times.

For the determination of chitinolytic activity, fungi were plated on 1.5% agar supplemented with either:

Table 1. Glycolytic activities in fungicolous fungi.

Fungus	Dissolution of				Mycoparasitic activity	
	cellulose-azure	chitin		lami-narin	number of spores/ml	% healthy conidio-phores*
		native	colloidal			
Tilletiopsis pallescens	−	−	−	+	10^6	1
T. minor B	−	−	−	+	10^6	5
Ampelomyces quisqualis	−	−	+	+	10^6	2
Sepedonium chrysospermum	−	−	+	+	10^6	3
Paecilomyces farinosus	−	+	+	(+)	10^6	5
Aphanocladium album	−	+	+	(+)	5×10^6	1
Verticillium lecanii II	−	+	+	+	5×10^6	0.5
V. psalliotae	−	(+)	+	+	5×10^6	12.5
Calcarisporium arbuscula	+	(+)	+	+	5×10^6	0.5
Acremonium alternatum	+	−	+	+	5×10^6	9
A. strictum	+	−	+	n.d.	5×10^6	13
Scopulariopsis brevicaulis	+	−	+	(+)	5×10^6	25
Peziza ostracoderma	+	−	−	−	5×10^6	50
Trichoderma viride	+	−	+	(+)	10^7	6
Penicillium chrysogenum	+	−	−	(+)	10^7	60
Sesquicillium candelabrum	+	−	+	n.d.	10^7	90

+ = positive reaction; (+) = positive reaction after preculturing; − = no clearance zone; n.d. = not determined.

* Assessed after application of mycoparasites to 10-day old cucumber powdery mildew colonies and rewetting 4 days later (Hijwegen, 1988).

a.) 1% native crab shell chitin (Sigma practical grade, milled in a ball mill for 6 h), 0.1% K_2HPO_4 and 0.1% $MgSO_4.7H_2O$, pH 8.0 (Veldkamp, 1955), or
b.) 0.5% colloidal chitin (prepared by the method of Godoy et al., 1982), 0.1% K_2HPO_4 and 0.1% $MgSO_4.7H_2O$, pH 7.7.

In both cases a clearance zone was taken to indicate the presence of chitinase activity. The experiment was repeated three times.

For the determination of β-(1,3)-glucanolytic activity fungi were plated on 1.5% agar supplemented with 1% laminarin (purchased from Koch-Light Ltd., Colnbrook, UK). Active fungi gave a clearance zone. The experiment was repeated twice.

There seems to be no relationship between the capacity to degrade cellulose-azure and the ability to control powdery mildew. Of the eight fungi giving the best performance against cucumber powdery mildew (Hijwegen, 1988) only *Calcarisporium arbuscula* showed detectable cellulolytic activity (Table 1).

This result strongly suggests that the capacity to produce cellulase is of no importance in this case.

With the exception of the two *Tilletiopsis* species, *Peziza ostracoderma* and *Penicillium chrysogenum*, all the fungi examined dissolved colloidal chitin. Native chitin, however, was only degraded by the three entomopathogenous fungi *Aphanocladium album*, *Paecilomyces farinosus* and *Verticillium lecanii*. Two of the other fungi

studied, viz. *Calcarisporium arbuscula* and *V. psalliotae* were capable of degrading native chitin only if they had been first precultured on colloidal chitin.

Four fungi which were very active in parasitizing cucumber powdery mildew (both *Tilletiopsis* spp., *Ampelomyces quisqualis* and *Sepedonium chrysospermum*) degraded laminarin rapidly (in 1-5 days) when transferred from malt extract agar to laminarin-containing agar.

Paecilomyces farinosus and *Aphanocladium album* did not dissolve laminarin when transferred directly from malt agar to laminarin-containing agar, but did so rapidly after being precultured on native chitin. These findings are similar to those published by Ordentlich et al. (1988), who demonstrated that *Serratia marcescens* only produced β-(1,3)-glucanase when both chitin and β-(1,3)-glucan were present.

Of the three fungi giving a poor performance against cucumber powdery mildew, *Scopulariopsis brevicaulis* and *Penicillium chrysogenum* degraded laminarin very slowly only after being precultured on 'cucumber powdery mildew'-agar (agar with 0.25% autoclaved spores of cucumber powdery mildew) or *Cladosporium fulvum* cell walls (prepared according to Ayers et al., 1976). *Peziza ostracoderma* did not dissolve laminarin even under these circumstances. The other fungi studied had intermediate levels of activity.

These results suggest β-(1,3)-glucanase to be the most important of the investigated cell wall-degrading enzymes with respect to mycoparasitism towards cucumber powdery mildew.

These experiments also suggest that preculturing mycoparasites on β-(1,3)-glucan containing substrates could trigger cell-wall degrading enzyme systems.

Acknowledgements

My sincere thanks are due to Dr Matthieu Joosten for providing the cell walls of *Cladosporium fulvum* and giving advice on the β-(1,3)-glucanase experiment and to Dr Nadia Danhash for correcting the English text.

References

Ayers, A.R., Ebel, J., Valent, B. & Albersheim, P., 1976. Host-pathogen interactions. X. Fractionation and biological activity of an elicitor isolated from the mycelial walls of *Phytophthora megasperma* var. *sojae*. Plant Physiology 57: 760-765.

Boekhout, T., 1991. Systematics of ballistoconidia-forming fungi. Ph.D. Thesis Utrecht, 109 + 194 pp.

Godoy, G., Rodrigues-Kabana, R. & Morgan-Jones, G., 1982. Parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi isolated from cysts of *H. glycines*. Nematropica 12: 111-119.

Hijwegen, T., 1988. Effect of seventeen fungicolous fungi on sporulation of cucumber powdery mildew. Netherlands Journal of Plant Pathology 94: 185-190.

Hijwegen, T., 1989. Effect of culture filtrates of seventeen fungicolous fungi on sporulation of cucumber powdery mildew. Netherlands Journal of Plant Pathology 95, Suppl. 1: 95-98

Ordentlich, A., Elad, Y. & Chet, I., 1988. The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phytopathology 78: 84-88.

Thompstone, A. & Dix, N.J., 1985. Cellulase activity in the Saprolegniaceae. Transactions of the British Mycological Society 85: 361-366.

Veldkamp, H., 1955. A study of the aerobic decomposition of chitin by microorganisms. Mededelingen van de Landbouwhogeschool Wageningen 55: 127-174.