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INHIBITION OF FUNGI AS AFFECTED BY OXALIC ACID PRODUCTION BY *SCLEROTIUM DELPHINII*

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Abstract

Sclerotium delphinii was found to secrete oxalic acid in the medium inhibiting growth of fungi. Conidia of *Trichoderma* spp. produced inflated vesicles and showed lysis whereas *Gliocladium roseum*, *Fusarium solani*, *F. oxysporum*, *Coniothyrium minitans*, *Botrytis cinerea* and *Mucor* sp. did not germinate at all. The metabolite produced by *S. delphinii* may be important in competition with other soil micro-organisms.

Introduction

Sclerotium delphinii Welch., a species related to *Sclerotium rolfsii* Sacc., is widespread in different parts of the world and causes economic losses in many crops (Aycock, 1966). During our studies on the interaction of *S. delphinii* with other fungi, the hyphae of *Trichoderma hamatum* approaching *S. delphinii* cultures showed some abnormal branching and swelling (Fig. 1). The kind of the metabolites secreted by *S. delphinii* affecting growth of *T. hamatum* was therefore examined.

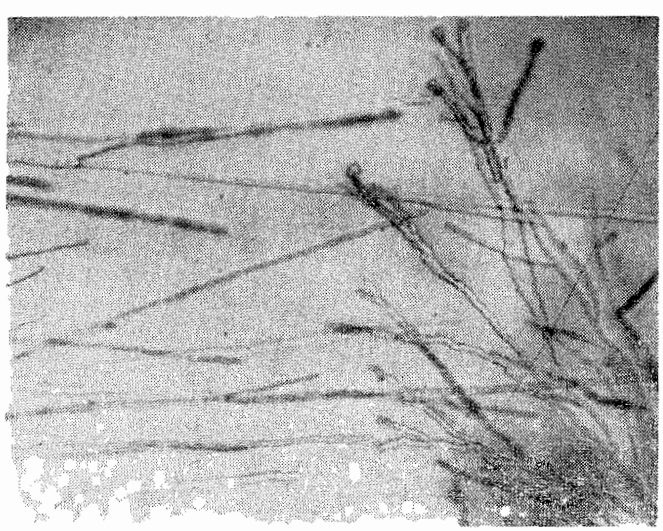

Fig. 1. Photomicrograph (x250) showing swelling and abnormal branching of the hyphae of *Trichoderma hamatum* (right) approaching hyphae of *Sclerotium delphinii* (left).

The isolate of *S. delphinii* used in this study was J40 present in the culture collection of the Botany Department, The University, Hull. This was previously isolated from crown rot of Iris by Dr. G.H. Boerema of the Plantenziekten Kundige Dienst, Wageningen, The Netherlands. *T. hamatum* was isolated from dried sclerotia of *S. delphinii* buried in the field (Coley-Smith et al, 1974).

Experimental Results

Using Heatley's (1947) agar plate method, a 9 cm diam. sterile sheet of cellophane was aseptically placed on plates of Oxoid PDA and inoculated with 10 mm disk of an actively growing *S. deplhinii* culture. After 4 and 10 days growth at 25°C, the cellophane sheet and the adhering fungus was removed. The metabolite which diffused through the cellophane into the agar medium was tested for its antifungal activity by studying the growth of *T. hamatum* on the medium. Controls were kept without *S. delphinii*. In plates where *S. delphinii* was grown for 4 days. *T. hamatum* did not grow for the first 24 hr., after which it showed a slow growth of 17 mm a day as compared to 26 mm a day in the control series. There was no growth of *T. hamatum* on plates where *S. delphinii* had grown for 10 days.

In another experiment, the antifungal activity of diffusates from *S. delphinii* was assessed by germinating conidia of *T. hamatum*. Ten mm disks of agar from a 10 day old *S. delphinii* culture were aseptically removed into sterile Petri dishes and a spore suspension in water containing conidia of *T. hamatum* was put on its surface. On the control disks, the conidia germinated within 18-20 hrs into 150-200 μm germ tubes whereas on the disks containing diffusates of *S. delphinii*, the conidia of *T. hamatum* produced an inflated vesicle with eventual lysis (Fig. 2). Of the other

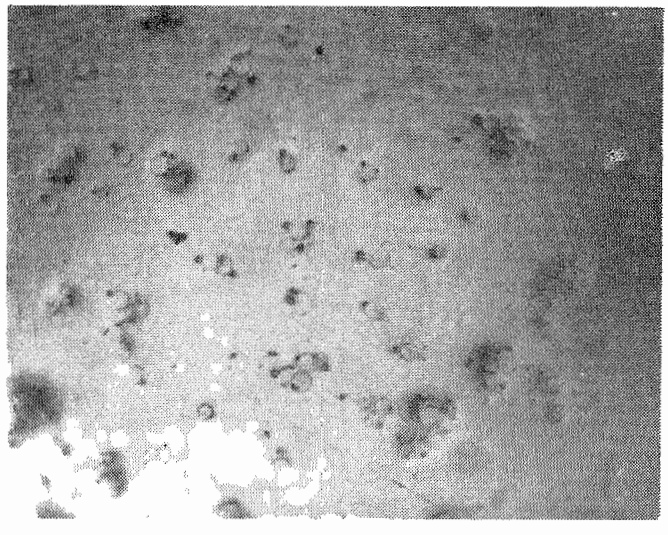

Fig. 2. Photomicrograph (x250) showing conidia of *Trichoderma hamatum* producing inflated vesicles and lysis.

fungi isolated from the sclerotia of *S. delphinii* (Coley-Smith et al. 1974), *T. harzianum*, *T. koningii*, *T. pseudokoningii*, and *T. viride* produced inflated vesicles and showed lysis like *T. hamatum*. Conidia of *Gliocladium roseum*, *Fusarium solani*, *F. oxysporum*, *Coniothyrium minitans*, *Botrytis cinerea* and aplanospores of *Mucor* sp. however, did not germinate.

There are reports of the production of oxalic acid by *S. rolfsii* (Higgins, 1927, Bateman & Beer, 1965; Bagyaraj & Sarsi, 1965) and *S. delphinii* (Perlman, 1948). The production of oxalic acid by *S. delphinii* and its effects on germination of fungal spores was therefore investigated. Twenty ml of Czapek Dox solution and potato

TABLE. 1. Effect of different media on the growth of *Sclerotium delphinii*, change in pH, oxalic acid content and antifungal activity of the culture filtrate at 25°C.

Media	DAYS					
	0	2	4	6	8	10
	Average Dry Weight of mycelium (mg)					
Czapek Dox solution	0	56	72	147	127	103
Potato dextrose broth	0	119	216	286	307	298
	Change in pH					
Czapek Dox Solution	5.3	2.8	2.4	2.4	2.3	2.3
Potato dextrose broth	5.5	2.6	2.3	2.2	2.0	2.0
	Oxalic acid (mg per ml.)					
Czapek Dox Solution	0	0.45	0.9	1.08	1.08	1.08
Potato dextrose broth	0	1.53	2.25	2.88	3.24	3.24
	Antifungal activity					
Germination of conidia in Czapek Dox Solution						
T. hamatum	+	+	+	—	—	—
T. viride	+	+	+	+	+	+
Germination of conidia in Potato dextrose broth						
T. hamatum	+	—	—	—	—	—
T. viride	+	+	—	—	—	—

+ = Conidia germinated.
— = No germination.

dextrose broth were separately dispensed in 100 ml Erlenmeyer flasks and inoculated with 10 mm agar disk from actively growing edge of *S. delphinii* culture. The fungus was grown as still cultures at 25°C and after 2,4,6,8 and 10 days interval the mycelial mat was harvested. The mycelial free filtrate were stored at 0°C and all the samples were analyzed for oxalic acid content using the $KMnO_4$ titration procedure described by Bateman & Beer (1965). Dry weight of the mycelium, change in the pH of filtrate, oxalic acid content and its antifungal activity as exhibited by the inhibition of germination of *Trichoderma* conidia are given in Table 1.

There was a considerable shift in the pH the medium. Oxalic acid upto 3.24 mg per ml of the filtrate was detected after 10 days growth in potato dextrose broth as compared to 1.08 mg per ml in Czapek Dox solution. Apparently the inhibition of germination of *T. hamatum* and *T. viride* conidia was related to the amount of oxalic acid in the medium.

For comparison, the effect of pure oxalic acid on the germination of conidia of *T. hamatum* and *T. viride* was examined. Oxalic acid dissolved in Czapek Dox solution and potato dextrose broth was used. Considering a decline in the pH of the media containing oxalic acid, controls were kept in which only pH of the medium was adjusted using H_3PO_4. In media containing oxalic acid @1.0 mg per ml. the conidia of *T. hamatum* did not germinate but produced an inflated vesicle and lysis (Table 2). At lower concentrations, though the conidia germinated but there was a considerable reduction in the size of the germ tube as compared to the control. *T. viride* appeared to be more tolerant to oxalic acid, germination being inhibited in 2.0 mg of oxalic acid per ml of the medium.

TABLE. 2. Effect of pure oxalic acid on the germination of conidia of *Trichoderma hamatum* and *T. viride* after 20 hours at 25°C.

	Concentration of oxalic acid (mg per ml)							
	0	0.25	0.50	1.0	1.5	2.0	2.5	3.0
Czapek Dox broth pH	5.3	2.4	2.2	2.0	1.9	1.8	1.8	1.8
Germination of conidia in Czapek Dox broth								
T. hamatum	+	+	+	–	–	–	–	–
T. viride	+	+	+	+	+	–	–	–
Potato dextrose broth pH	5.5	4.2	3.6	3.0	2.7	2.4	2.3	2.1
Germination of conidia in Potato dextrose broth								
T. hamatum		+	+	–	–	–	–	–
T. viride	+	+	+	+	+	–	–	–

+ = Conidia germinated.

– = No germination

Conclusion

Sclerotium delphinii was found to secrete oxalic acid in the medium. Production of oxalic acid in the culture was observed from 2 days onward. The production of certain metabolites is known to favour the producer in its competition with other organisms. The results of the present investigation indicate that oxalic acid produced by *S. delphinii* may play an essential role in its competition with other soil fungi affecting its survival. The production of oxalic acid by *S. delphinii* and its accumulation in soil inhibiting growth of soil micro-organisms need further investigation.

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