SHORT COMMUNICATIONS

The Relationship between Early Hyphal Branching and Formation of Sclerotia in *Sclerotium rolfsii*

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(Received 8 March 1973; revised 11 June 1973)

The morphological process of sclerotium formation in *Sclerotium rolfsii* (*Corticium rolfsii*) was described in detail by Goujon (1970), who noted that the mycelium of this fungus was composed mainly of leading hyphae with internodes 240 μ m long containing 40 nuclei/cell, and lateral hypha with 10 nuclei/cell. Lateral branches first emerge from the leading hyphae at acute angles; later, thinner branches emerge at right angles and give rise to sclerotia. The effects of nutritional, chemical and physical factors on sclerotium formation in *S. rolfsii* have been studied (Wheeler & Waller, 1965; Henis, Chet & Avizohar-Hershenzon, 1965; Chet, Henis & Mitchell, 1966; Okon, Chet & Henis, 1972). Whereas these authors agree that partial inhibition of linear growth is often followed by sclerotium formation, no attention has hitherto been paid to the possible effect of chemicals on the morphology and growth pattern of the hyphae.

The present paper describes the effect of various substances which influence sclerotium production on the branching process of the leading hyphae at the colony margins, as related to formation of sclerotia in *Sclerotium rolfsii*.

METHODS

Types R and A of *Sclerotium rolfsii* Saccardo (Chet & Henis, 1972) were grown at 30 °C in Petri dishes (8.5 cm diam) containing 15 ml of the synthetic medium (SM) of Okon, Chet & Henis (1973). The plates were inoculated in the centre with agar discs (0.5 cm diam) covered with fungal mycelium, which had been cut from the edge of a 5-day-old colony. There were five replicates of each treatment.

Lactose, iodoacetic acid, ethanol, sodium acetate, L-cysteine and L-threonine, were all Analytical Reagent grade. Tenfold concentrated solutions were sterilized by filtration through a 0.45 μ m HA Millipore filter and added aseptically to the melted agar.

RESULTS

Quantitative data on the effect of various chemicals on the morphological appearance of the hyphae at the colony margins and on the formation of sclerotia are given in Table 1.

With the exception of lactose with type A, all substances significantly inhibited development of the first and second branch in types A and R of *Sclerotium rolfsii*. However, this inhibition was less significant with substances that increased formation of sclerotia, i.e. lactose, iodoacetic acid and L-threonine. These substances also shortened the internodes considerably, thus giving rise to more lateral branches per unit length of the hyphae, as compared with the control. Internode length was much less affected by ethanol and L-cysteine,

Table 1. *Effect of some substances on sclerotium production and morphology of Sclerotium rolfsii*

Medium	Fungal isolate	Colony diam (mm)		No. and location of sclerotia	Morphology of hyphae at the colony margins after 72 h: Length* of		
		3 days	8 days		Internode (μm)	2nd branch (μm)	1st branch (μm)
SM	R	85	85	125† (6) CM	375 a‡	285 a	120 a
	A	85	85	500 (4) D	375 a	285 a	120 a
SM+lactose (0.5 %)	R	26	55	200 (4) CR	210 b	185 b	95 a
	A	85	85	500 (4) D	375 a	285 a	120 a
SM+ethanol (2.0 %, v/v)	R	20	85	0	375 a	125 c	30 c
	A	45	85	200 (6) CC	295 a	100 c	15 c
SM+sodium acetate (0.1 %)	R	37	85	30 (8) CM	90 c	70 c	0 d
	A	37	85	80 (3) CR ₄	100 c	80 c	0 d
SM+iodoacetic acid (5×10^{-5} M)	R	17	35	70 (4) CR ₂	50 d	120 c	60 b
	A	50	85	90 (4) CR ₃	60 d	100 c	45 bc
SM+L-cysteine (10^{-2} M)	R	32	80	0	225 ab	135 c	30 c
	A	55	80	0	225 ab	135 c	30 c
SM+L-threonine (10^{-2} M)	R	22	50	150 (3) CR ₂	125 c	135 c	45 bc
	A	35	83	1000 (2) AC	125 c	135 c	45 bc

* Diagram of hyphal branching in colony margins:

† Time of appearance (days) is shown in brackets. CM, colony margins; D, dispersed over the colony; CC, colony centre; AC, colony densely covered with sclerotia; I, initials only; CR_{1, 2, 3, 4}, circles of sclerotia.

‡ Each number represents the mean of measurement of 50 hyphae taken from five plates. Numbers accompanied by the same letter in each column do not differ significantly at the 5 % level of probability using Duncan's multiple range test.

whereas sodium acetate shortened internode length but also very strongly inhibited branch development.

In addition to their effect on branch development and internode length, the substances tested also affected the gross morphology of the hyphae. Thus, when grown on SM the colony margins of types R and A consisted mainly of leading hyphae; in both types iodoacetic acid induced the formation of rhizomorph-like hyphae. When grown in the presence of L-threonine, the colony margins consisted mainly of lateral hyphae, whereas sodium acetate caused the formation of very dense leading aerial hypha.

In general, substances which inhibited sclerotium formation favoured the production of aerial hyphae, whereas induction of sclerotium formation was preceded by extensive development of both aerial and agar-penetrating hyphae.

DISCUSSION

Formation of sclerotia and the branching pattern at the colony margins appear closely related in *Sclerotium rolfsii*.

Plunkett (1966) suggested that the effect of different amino acids on branching in *Mucor hiemalis* arose through the different proteins formed. Fevre (1972) found that the addition

of puromycin, mitomycin C or *p*-fluorophenylalanine completely inhibited lateral branching of *Saprolegnia monoica*, with a concomitant reduction in the activity of some hydrolytic enzymes (cellulase, glucanase) possibly involved in initial stages of branching. Partial inhibition of linear growth is an important condition (Wheeler & Waller, 1965), but not the only condition for sclerotium formation (Okon *et al.* 1972). On the other hand, substances such as ethanol and acetate that preferentially inhibit development of lateral branches also inhibit sclerotium formation. Although branching seems to be related to sclerotium formation in both types, the 'efficiency' of a mycelial unit in producing sclerotia is higher in type A than in type R (Chet & Henis, 1972). However, no differences in their branching patterns at the colony margins could be observed. This suggests that formation of aerial hyphae and lateral branching may not be the only factors that govern sclerotium formation.

We have demonstrated that some substances, including sodium acetate and ethanol, gave rise to superficial leading hyphae, whereas iodoacetic acid, lactose and L-threonine favoured mycelial growth within the agar medium. No explanation can be offered at present for these different growth patterns.

The relationship between branching and sclerotium formation may be considered as part of the basic problem of branching control in fungi (Robertson, 1965). If apical dominance by hormone-like substances is involved in the repression of lateral branching (Robertson, 1965), a similar regulation mechanism may function in the control of sclerotium formation (Chet & Henis, 1968). Hypothetical repressors of sclerotium formation may be produced by the growing hyphal tip and move backwards. On the other hand, the theory of trophic competition between hyphae may hold here (Robertson, 1965). In either instance, growth arrestment of the leading hyphae will promote branching and sclerotial formation.

The results offer an explanation for the enhancement of translocation activity observed in *Sclerotium rolfii* before sclerotium formation (Okon *et al.* 1973). Translocation of labelled compounds from the colony centre towards its margins was prevented when the fungus was grown in the presence of ethanol, and was enhanced by lactose, before the appearance of sclerotial initials. These differences in translocation correspond to the degree of lateral branching at colony margins of *S. rolfii*. We suggest, therefore, that translocation activity in *S. rolfii* depends on the number of lateral hyphal tips, which function as 'active metabolic pumps' and are probably more efficient in creating chemical gradients than are the tips of the leading hyphae. The higher rate of translocation in *S. rolfii* colonies and preferential uptake of metabolites by sclerotial initials (Okon *et al.* 1973) and of ^{32}P by sclerotia (Wilcoxon & Subbarayudu, 1968) possibly reflect extensive branching at these sites, and may be a secondary rather than a primary factor in sclerotium formation.

This investigation was supported by a grant from the 'Histadrut' Israel General Workers' Organization.

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