The Response of Two Types of *Sclerotium rolfii* to Factors Affecting Sclerotium Formation

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SUMMARY

Ten isolates of *Sclerotium rolfii* were divided into two types (R and A) which differed in their response to factors affecting sclerotium formation. It is suggested that differences in carbohydrate metabolism may account for the differences between them.

INTRODUCTION

Many chemical and physical factors affect sclerotium formation in *Sclerotium rolfii* Sacc. (Townsend & Willetts, 1954; Chet, Henis & Mitchell, 1966; Chet & Henis, 1968). Henis & Chet (1968) suggested that some of those involved in initiation, development and maturation of the sclerotia are of mycelial origin. However, no comparative information is available on the responses of various isolates of *S. rolfii* to such factors.

METHODS

Ten isolates of *Sclerotium rolfii* Sacc. from infected sugar beet, bean and potato plants were grown on a synthetic medium composed of 4 % (w/v) glucose, 0.02 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 % K_2HPO_4 , 0.02 % KCl, 0.1 % NH_4NO_3 , 0.0001 % thiamine hydrochloride, 0.0002 % Fe^{2+} , 0.0002 % Mn^{2+} , 0.0002 % Zn^{2+} and 2 % (w/v) Bacto agar (pH 6.8) and incubated at 30 °C. Each experiment was carried out with five replicates.

RESULTS AND DISCUSSION

The isolates tested were divided into two types which differed in their morphological and physiological properties (Table 1). Six isolates (collectively designed type R) resembled the one used in our previous studies (Chet *et al.* 1966), having a thick mycelium producing 80 to 100 sclerotia/plate, mostly near the plates' walls, after 6 to 7 days of incubation (Fig. 1*a*).

The remaining four isolates (collectively designed type A) had a sparse mycelium and, after 72 h, produced 500 to 600 sclerotia/plate, mainly in a band 18 mm wide, towards the periphery of the plate (Fig. 1*b*). Hyphal-tip cultures were prepared from every isolate for three successive generations. All the isolates preserved their original characteristic morphology. When colonies of both types were grown together they showed a mutual growth inhibition (Fig. 1*c*). When mycelium was transferred from the region of their contact, new types were not observed, suggesting no anastomoses formed between those types.

Although having a similar growth rate at pH 6.8, type A was more tolerant than type R to low pH values (Fig. 2) and its ability to form sclerotia was not affected, whereas type R

Fig. 1. Type R (a) and A (b) grown on a synthetic medium for 8 days at 30 °C. (c) Types R (top) and A (bottom) grown together on the same plate. (d) The effect of 0.2 % 2-deoxy-D-glucose on type R (type A was identical with control).

produced only few sclerotia (10 to 15/plate) at extremely low (pH 3 to 4) or high (pH 9 to 10) pH values. These types also differed in their response to compounds previously reported to affect sclerotium formation in *Sclerotium rolfsii*. Thus, sclerotium formation in type R was induced by iodoacetic acid (Chet *et al.* 1966) and by lactose (Okon, Chet & Henis, 1972) and inhibited by cysteine (Chet *et al.* 1966), and by ethanol (Henis, Okon & Chet, 1971), whereas the new type A was not affected by these compounds. When the lactose concentration was increased to 1.5 % (w/v) and the glucose concentration was decreased to 0.5 % (L+G medium), mycelial growth and sclerotium formation of type R were inhibited, while type A continued to produce about 500 sclerotia/plate in a wide circular area. When 2-deoxy-D-glucose, a known antimetabolite of glucose in polysaccharide synthesis (Johnson, 1968), was

Fig. 2. Growth of type A (●—●) and R (○—○) on synthetic medium (see text), adjusted to pH 3 with HCl. The medium was prepared as a concentrated liquid and autoclaved separately from the agar. After cooling, the medium and agar were mixed.

Table 1. *The effect of various treatments on Sclerotium rolfsii strains (examined after 15 days)*

Treatment	Type A	Type R
Cysteine (10^{-2} M)	Sclerotial initials present	No sclerotia
Iodoacetic acid (10^{-4} M)	'Feather-like' mycelium, randomly spread sclerotia	Circle of sclerotia
Ethanol (2 to 4 %, v/v)	Sclerotia randomly formed	No sclerotia formed
G + L medium*	Sclerotia randomly formed	Circle of synchronous sclerotia formed
L + G medium†	Sclerotia formed in a circular area	Partially inhibited mycelium, no sclerotia formed
2-Deoxy-D-glucose: 0.2 to 0.6 %	No effect on sclerotium formation	Total inhibition of sclerotium formation
0.7 to 1.0 %	Partial inhibition of mycelial growth and sclerotium formation	Partial mycelial inhibition, total inhibition of sclerotia

* Synthetic medium containing 1.5 % (w/v) glucose and 0.5 % lactose (Okon *et al* 1972).

† Synthetic medium identical with G + L but containing 1.5 % (w/v) lactose and only 0.5 % glucose.

added to the synthetic medium it completely inhibited sclerotium formation in type R but did not so affect type A (Table 1), suggesting that the two types differed in their carbohydrate metabolism. Aycock (1966) mentioned the presence of different morphological types of *S. rolfsii*. Our observations lead to the conclusion that the differences between type R and A were not only morphological, because sclerotium formation in type A, unlike that in type R, was not affected by the compounds tested. Furthermore, whereas growth and sclerotium formation in type R were independently affected, no such difference between the response of growth and sclerotium formation could be observed in type A. It is therefore possible that a control mechanism of sclerotium formation exists only in type R. A study of the physiological basis of this difference may lead to a better understanding of this mechanism in *S. rolfsii*.

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