Lactose-induced Synchronous Sclerotium Formation in *Sclerotium rolfsii* and Its Inhibition by Ethanol

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SUMMARY

Synchronous formation of sclerotia in *Sclerotium rolfsii* was induced in synthetic media containing glucose (0.3 to 1.9%, w/v) by adding 0.5% (w/v) lactose. Sclerotia were formed after 4 days at the colony margin in a circular band, the width of which was directly related to glucose concentration. Sclerotium formation in media containing glucose, with and without lactose, was completely inhibited by the addition of 2% (v/v) ethanol. This inhibition was not affected by iodoacetic acid (5×10^{-6} M). Neither lactose nor ethanol was oxidized by the fungus. Oxygen consumption with glucose as substrate was partially inhibited by ethanol but not by lactose. The significance of this system in morphogenetic studies is discussed.

INTRODUCTION

Sclerotium formation in *Sclerotium rolfsii* Sacc. on agar plates usually occurs at different times within the colony. Synchronization of formation is essential for the study of structural and biochemical changes preceding and accompanying sclerotium formation.

Nutritional and mechanical factors involved in sclerotium formation were studied by Henis, Chet & Avizohar-Hershenzon (1965) and by Wheeler & Waller (1965). According to Chet, Henis & Mitchell (1966), sclerotium production in *Sclerotium rolfsii* is prevented by sulphur-containing amino acids and induced by iodoacetic acid. Chet & Henis (1968) suggested that iodoacetic acid induced sclerotium formation by the inactivation of a hypothetical intracellular repressor. B. E. J. Wheeler (personal communication, 1968) found that low concentrations of ethanol interfered with sclerotium formation.

In this paper, a system which induces the synchronous formation of sclerotia in *Sclerotium rolfsii* is described, together with some inhibitory effects of ethanol on sclerotium formation.

METHODS

Fungal strain and growth medium. *Sclerotium rolfsii* Sacc. isolated from sugar beet, was grown on a synthetic medium of Joham (1943) as previously described (Chet *et al.* 1966).

Agar discs (0.5 cm diam.), covered with fungal mycelium, were cut from the edge of a 5-day-old colony and used as an inoculum. All cultures were incubated at 30 °C. There were always five replicates of each treatment.

Alcohol dehydrogenase activity. *Sclerotium rolfsii* was grown on a cellophane membrane placed on plates of Joham's agar. The mycelium was homogenized with 10 ml of 0.05 M-tris-HCl buffer (pH 8.5) in an Omnimixer (Sorvall) at 800 rev./min for 2 min and then in an ultrasonic disintegrator (MSE) at 1.5 A for 5 min. Debris was removed by centrifuging at 10000g for 15 min.

Fig. 1

Fig. 1. Effect of glucose concentration on linear growth of mycelium of *Sclerotium rolfsii* in the presence of 0.5% (w/v) lactose. Glucose concentrations (% w/v): $\triangle$ — $\triangle$, 1.9; $\blacktriangle$ — $\blacktriangle$, 1.5; $\circ$ — $\circ$, 1.1; $\bullet$ — $\bullet$, 0.7; $\square$ — $\square$, 0.3.

Fig. 2

Fig. 2. Effect of glucose concentration on the number of sclerotia formed by *Sclerotium rolfsii* on a lactose-containing medium. (Lactose concentration, 0.5% (w/v); incubation time, 9 days.)

The enzyme was assayed by the method of Zink (1969). Protein content was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine albumin as a standard. One unit of enzyme was defined as the amount of enzyme causing a change of 0.001 absorbance units/min. Specific activity was determined as the number of units/mg protein in solution.

Saccharomyces oviformis (obtained from the Wine Institute, Rehovot, Israel) was used as a reference organism for the enzyme assay. It was grown for 7 days on yeast dextrose agar (Difco), then harvested and homogenized as already described.

Oxygen uptake. The fungus was grown in 250 ml (Erlenmeyer) flasks containing 75 ml Joham's liquid medium on a shaker (100 strokes/min) for 3 days. The mycelium was collected by centrifugation at 800g for 10 min, then washed five times in 0.01 M-phosphate buffer (pH 7.0), also by centrifugation. *Sclerotium rolfsii* was starved in the same buffer for 24 h at 5 °C. Oxygen uptake of 0.2 ml samples containing about 30 mg dry weight was measured in a Gilson differential respirometer (100 strokes/min) at 30 °C. The reaction mixture (2.2 ml total volume) contained phosphate buffer (pH 7.0), substrates and fungal mycelium.

RESULTS

Effect of lactose on linear growth and sclerotium formation. The fungus did not grow on the synthetic medium with lactose (0.5 to 2%, w/v) as the sole carbon source. With glucose (0.5 to 2%, w/v) as the sole carbon source the fungus grew well and sclerotia began to form after 6 days. When both glucose and lactose were added to the medium sclerotia formed synchronously in a band at the edge of the colony after 4 days. In the medium with 0.5% lactose, mycelial growth (Fig. 1) and the numbers of sclerotia (Fig. 2) increased with glucose concentration and the width of the sclerotial band was directly related to glucose concentration (Fig. 3). Little variation was observed between replicates.

Fig. 3. Effect of glucose concentration on diameter of sclerotial circle on lactose-containing medium.

Inhibition of sclerotial formation by ethanol. Ethanol (2.0 to 4.0%, v/v), added to Joham's synthetic medium, temporarily slowed mycelial linear growth and completely inhibited sclerotium formation (Fig. 4, 5).

Ethanol (2%, v/v) also inhibited sclerotium formation on Joham's medium supplemented with 5×10^{-5} M-iodoacetic acid which, as previously reported (Chet *et al.* 1966), induced sclerotia to form in several circles around the point of inoculation. Ethanol also inhibited the synchronous formation of sclerotia on lactose-supplemented media (Fig. 6, 7).

Ethanol dehydrogenase activity in mycelial extract. Alcohol dehydrogenase activity could not be detected in extracts of the fungal mycelium grown for 4 and 14 days on Joham's agar, whereas comparable extracts of 7-day-old cultures of *Saccharomyces oviformis* showed a specific activity of 350.

Oxygen uptake. The effects of lactose, ethanol and glucose on respiration of *Sclerotium rolsii* are summarized in Fig. 8. Lactose and ethanol were not utilized as sole carbon sources for exogenous respiration. Oxygen uptake with glucose as a substrate was affected by ethanol but not by lactose.

DISCUSSION

Sugars and sugar alcohols affect differentiation in *Physarum polycephalum* (Chet & Rusch, 1969), *Myxococcus xanthus* (Dworkin & Gibson, 1964) and *Sclerotinia sclerotiorum* (Liu-Su Chin & Le Tourneau, 1970). Both timing and location of sclerotial formation in *Sclerotium rolsii* could be controlled by changing glucose concentration in lactose-supplemented Joham's media. Chet & Henis (1968) suggested that sclerotium formation in *S. rolsii* was induced by inactivation of a $-SH-Cu^{+2}$ protein which acts as a repressor of sclerotium formation. It might be postulated that ethanol could increase the level of $-SH$ groups by entering the metabolic pathway through the $-SH$ -containing alcohol dehydrogenase (Fruton & Simmonds, 1958). In that case it should not have affected sclerotium formation in the presence of iodoacetic acid (which inhibits alcohol dehydrogenase activity). Furthermore,

Fig. 4. *Sclerotium rolfii* grown on synthetic medium (control).

Fig. 5. Inhibition of sclerotium formation by ethanol (2 %, v/v).

Fig. 6. Synchronous formation of sclerotia in the presence of lactose (0.5 %).

Fig. 7. Inhibition of sclerotium formation by ethanol in the presence of lactose (0.5 %).

Fig. 8. Respiration of *Sclerotium rolfsii* on lactose, glucose and ethanol. ▲—▲, 2 % Glucose; △—△, 2 % glucose + 2 % lactose; □—□, 2 % glucose + 3 % ethanol; ●—●, 3 % ethanol; ○—○, 2 % lactose.

alcohol dehydrogenase activity was not found in the mycelium, nor could ethanol serve as a substrate for oxygen uptake by *S. rolfsii*.

Another phenomenon which seems incompatible with the sulphhydryl hypothesis is the effect of lactose on sclerotium formation. Thus, the -SH level of the mycelium is probably not the sole factor which directly controls sclerotium formation. A more specific control mechanism of morphogenesis in *Sclerotium rolfsii* should therefore be sought.

According to Wheeler & Waller (1965) initiation of sclerotia in *Sclerotium rolfsii* was regulated by the growing hyphae. They noted that initials did not appear until the lateral extension of mycelium was restricted. Henis *et al.* (1965) also found that sclerotia were produced along a line cut in the mycelium. Both findings indicate that partial inhibition of linear growth is required for sclerotium formation. However sulphur-containing amino acids (Chet *et al.* 1966) and ethanol both partially inhibited linear growth of *S. rolfsii* and also inhibited sclerotium formation. Similarly, cycloheximide inhibited linear growth of *S. rolfsii* when applied in strips or cylinders at colony margin without inducing sclerotium formation (Chet, 1967). Thus, the relationship between partial inhibition of linear growth and sclerotium formation in *S. rolfsii* remains unsolved. Although the modes of action of lactose and ethanol on morphogenesis of *S. rolfsii* are not yet known, this new system of synchronizing and inhibiting formation of sclerotia may prove useful in further studies.

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