NOTE G. Kritzman et al. (1980) Phytoparasitica 8(1): 73-76.

GERMINATION-INHIBITOR IN *BOTRYTIS ALLII* SPORES

By

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Spore germination of *Botrytis allii* was influenced by the spore concentration and the viscosity of the germination medium. A method was developed for spore germination of *B. allii* in a medium without nutrients. Evidence was obtained of the existence of a germination-inhibiting factor which is produced by the germinating cultures. This inhibitor is thermostable and has a low molecular weight.

KEY WORDS: Spore germination; spore germination inhibitor.

The process of spore germination of *Botrytis* spp. has been studied by Hawker (12) and McKeen (17). The germination occurs in a wide range of temperatures (9, 11). *Botrytis* spores require high humidity (93-100% R.H.) for germination (18, 23), but their ability to germinate in water was found to be very poor (3) or non-existent (2). Stimulation of the germination of *B. cinerea* in water was achieved by the addition of nutrients to water, *e.g.* sugars and amino acids (1, 2, 4, 5, 20, 21).

The existence of germination self-inhibitors is a well known phenomenon in some fungi (6, 7, 10, 15, 16, 24). However, very little attention has been paid to the inability of spores to germinate in water. The aims of the present study were to (*a*) detect self-inhibitors in *B. allii*, and (*b*) develop a method for spore germination of *B. allii* in water.

Botrytis allii isolated from onion bulbs was grown at 24°C in petri dishes containing 15 ml of a basal medium prepared according to Kirtzman and Netzer (14). The plates were inoculated in the center with fungal mycelium cut from the edge of a 7-day-old colony.

Standard conidial suspensions were prepared by gentle washing of the surface of 21-day-old cultures with a 5-ml solution of Tween 20 in distilled water (1:2000, v/v). The spores were filtered through cheesecloth and centrifuged for 20 min at 20,000 x*g*. The supernatant fluids were discarded and the spore sediment was mixed with distilled water for studying spore germination. A suspension of 10^4 spores/ml was either spread on the surface

Publication of the Agricultural Research Organization. No. 137-E, 1980 series. Received Sept. 18, 1979; received in final form Jan. 22, 1980.

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of petri dishes containing water agar or put in a liquid medium containing various agar concentrations. The incubation time was 24 h.

A germinated spore was defined as one with a germinating tube equal to or longer than the spore box. Partial characterization of the inhibitor was accomplished by analyzing the supernatant fluid remaining after spore inoculation in water (10^9 spores/ml for 48 h).

The supernatant was heated at 121°C for 20 min to determine thermostability. The inhibitor was dialyzed against distilled water at 4°C for 48 h to determine its approximate molecular size. Total carbohydrates were determined with the anthron reagent (13), glucose contents by the glucose-oxidase method (Sigma), and total amino acids with the ninhydrin reagent (22).

The germination and germ tube elongation of *B. allii* spores were followed at the following temperatures (± 0.5°C): 15°, 20.5°, 25° and 30°C. At each temperature, 200 spores were tested in three replicates. The optimal temperature was found to be 25°C for both parameters.

A 25°C, 30%, 63% and 95% (± 3% S.E.) of the spores germinated after 3.5, 5.5 and 7.5 h of incubation, respectively. At the same times, the rate of germ tube elongation was 6.7, 5.5 and 4.3 mm^3/h, respectively. The same pattern was observed at the other temperatures tested. In these experiments the spores were spread on the surface of a water agar medium. However, when spores were placed in liquid (distilled water), no germination was observed. These spores, although they failed to germinate when transferred to distilled water and incubated for 48 h, did not lose their germination potential. This was proved by re-transferring them back to the surface of a water agar medium with 0.5% agar in distilled water.

An experiment was carried out to test the possibility of removing the self-inhibitor from the spores by washing them. The spores, at a high concentration, failed to germinate in water. It was concluded therefore that a high concentration of *B. allii* spores produced a factor which probably was released into the water during germination and had germination-inhibiting activity.

Two parameters were found to play an important role in spore germination (Table 1):

TABLE 1

EFFECT OF SPORE CONCENTRATION AND AGAR CONCENTRATION ON THE GERMINATION OF *BOTRYTIS ALLII* SPORES

(Means of 200 spores in each of five replicates; S.E. = 1.0%)

Spore age (weeks)	*Spore concentration (x 10^4 per ml)*	*Agar concentration (%, w/v)*	*Spore germination (%)*
3	1.0	0.05	95
4	1.0	0.05	98
3	1.0	0.10	67
3	1.6	0.20	10
3	1.6	0.10	50
3	1.6	0.05	92
4	1.6	0.05	85
3	1.6	0.01	5
4	3.2	0.05	50
3	3.2	0.05	53
3	8.0	0.05	10
3	8.0	0.10	0
3	8.0	0.20	0
3	16.0	0.05	0
4	16.0	0.05	0

spore concentration and the viscosity of the medium (expressed as percentage of agar in the water). A high percentage of germinating spores was achieved in media containing 0.05% agar (Noble) and with a spore concentration of 10^4/ml. The agar concentration could affect spore germination in at least two ways: by absorbing the inhibition factor, and by improving the aeration conditions.

Addition of fructose, glucose or galactose to the germination medium at a final concentration of 10^{-2} M did not increase the percentage of germinating spores in comparison with the results shown in Table 1.

There was no difference in behavior between 3- and 4-week-old spores (Table 1).

A medium containing the germination-inhibiting factor (obtained from 10^9 spores/ml) and 0.05% agar was lyophilized and its effect on spore germination was tested at various concentrations. Even dilution of the inhibitor to one-third of its original concentration, completely inhibited the germination of *B. allii* spores. At 1:4 dilution, 40% of the spores germinated; at 1:16, 70%; and at 1:32, 98%. The results revealed that the inhibitor is thermostable and has a low molecular weight, since it was dialyzable.

Amino acids (26 μg/ml) and 142 μg/ml sugars (containing 112 μg/ml glucose) were detected in the diffusate and might be associated with the inhibiting factor. The mode of action of the inhibitor and its nature are still unknown. However, it is evident that this factor is produced by the spores of *B. allii*, as reported by Brown (4, 5) and Schütt (19) for *B. cinerea*. Such an inhibitor serves as a regulator of spore germination in an environment with a high concentration of spores.

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