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Factors Affecting Spore Germination of *Volvariella volvacea*

By

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Abstract

Environmental factors affecting basidiospore germination were studied with *Volvariella volvacea,* the edible straw mushroom which is common in South East Asia. A relatively mild heat shock is necessary for spore germination. The spores give best germination at 40°C, although early hyphal growth is better at 35°C the germination of spores is affected by temperature, pH, a presoaking treatment and spore density. Higher pH supports more germination but seems unfavourable for early mycelial growth. Presoaking treatment in phosphate buffer solution or distilled water also stimulates germination markedly.

Introduction

The straw mushroom, *Volvariella volvacea* (Amanitaceae) — is extensively grown in the tropics and subtropics. It is an obligatively saprophytic fungus growing on decaying plants, especially the straws. Its use as a delicious food in China and South East Asia has been as common as is the use of the white mushroom *Agaricus* in western countries. The productive season in Hong Kong extends from May to October. However, cultivation in laboratory conditions will supply fruit bodies all the year round.

As noted by Singer (13), little is known of the biology of this fungus. Only their morphological characteristics, cultivation method, and nutritional contents have been studied (3, 4, 13). Therefore, we became interested in studying its physiology and cytology. The environmental factors affecting spore germination and a reliable method for spore collection and isolation are described and discussed here.

Materials and Methods

Fruit bodies of *Volvariella volvacea* were obtained from small beds grown in laboratory conditions by slight modification of the cultivation method described by Chang (3). The normal fruit bodies at mature stage, which means that the pileus had completely expanded and the gills were light pinkish white in colour, were collected, their stipes removed, and they were then hung inside the sterilized tall cylindrical glass jars. The spores were collected by placing Petri dishes with media or solutions under the fruit bodies, facing the gills. The time required for spore collection was usually from a few seconds to two minutes, depending on the desired spore density and the degree of maturation of the fruit body. With this method, spores could be scattered uniformly on the medium. A temperature of 20°C or above is necessary for the discharge of spores. In our experiments, the fruit body continued to discharge spores for about 18 hours after it had been placed in the jar at 25°C.

Complete medium was used throughout the experiment unless specifically stated. (Formula: $MgSO_4$ 0.5 g, KH_2PO_4 0.46 g, K_2HPO_4 1 g, peptone 2 g, dextrose 20 g, agar 20 g per litre of distilled water). The medium was sterilized and a layer 4–5 mm thick was poured onto the plastic Petri dishes.

Usually spores germinate after 16 hours incubation at 40°C, but counts on germination percentage were made after 40 to 48 hours. The criterion for germination is, in this experiment, the appearance of germ tubes. Plates in triplicate or quadruplicate were used in each experiment and the results were checked. Counts of 100 spores were made on three individual areas of each plate. Their averages are given in the data.

Single spores were cut out from the medium under a microscope by means of a spore cutter described by Raper (12). Isolation of the spores was accomplished by a needle which was blunted and sharpened uniformly on opposite sides by rubbing it against an oilstone. A small cylindrical block of agar containing the spore could then be lifted out and placed, one or ten, in a plate for germination.

Results and Discussion

Basidiospores of *V. volvacea* are usually asymmetrical and tend to be egg shaped. The average length is 7 to 9 μ, with the widest part 5 to 6 μ and the narrowest part 3–4 μ across (5). The wall is relatively thick and brown in colour when spores shed. Germination of spores are not observed in room temperature (22–25°C). Protrusion of the germ tube is always at the hilum, which is a slightly protruded part with thinner wall; it is also believed to be the point of attachment of the spore to sterigma. The germ tubes, protruding from the spores, usually extend to a certain length before branching, or occasionally they may branch at the germination point in bidirections. In some cases, germ tubes may remain unbranched even at a length of 835 μ. The germinated spores were mostly found with their cellular contents migrated to the hyphae, leaving an empty spore wall but spores with the nuclei remaining inside were also observed. Swelling of the spore, one of the criteria of germination for other fungal spores, was not observed, even after germ tube protrusion, and this may be due to the nature of the spore wall.

Effect of temperature. — Various temperatures were tried for spore ger-

Table 1. *Mean germination percentage and average germ tube length of soaked and unsoaked spores incubated at different temperatures and varying pH values.*

Treatment		Unsoaked spores (control)		Soaked spores			
				In distilled water		In phosphate buffer	
Temperature °C	pH	Germination %	Germ tube length μ	Germination %	Germ tube length μ	Germination %	Germ tube length μ
35	6.8	25.45	121.11 ± 8.75	83.00[1]	182.31[2] ± 11.37	89.45	234.73 ± 20.03
	7.5	40.91	77.27 ± 6.98	81.38	148.63 ± 7.61	79.50	127.25 ± 7.79
40	6.8	38.15	62.30 ± 4.59	84.77	193.66 ± 13.02	92.58	168.12 ± 16.40
	7.5	46.95	35.04 ± 2.82	85.84	100.95 ± 11.29	79.92	77.40 ± 7.46

[1] All mean germination percentages of spores pretreated in solutions exceed the 1 % level of significance compared with their controls, whereas two means from spores presoaked in distilled water and in phosphate buffer are never significantly different at the 1 % level.

[2] All means from presoaked spores exceed the 1 % level of significance compared with their controls. The differences between any two means, as regards two temperatures at same pH value and two pH values at same temperature (with the exception of the pair in water at pH 6.8), are also highly significant.

mination. Spores were placed at 20, 25, 30, 35, 40, 45°C for two days. No germination was observed at 20, 25 and 45°C in either complete or potato dextrose agar (Difco) medium. In complete medium very few spores germinated at 30°C and maximum germination took place at 40°C, whereas in potato dextrose agar medium the temperature range for germination was even smaller — spore began germination at 35°C and reached a maximum at 40°C

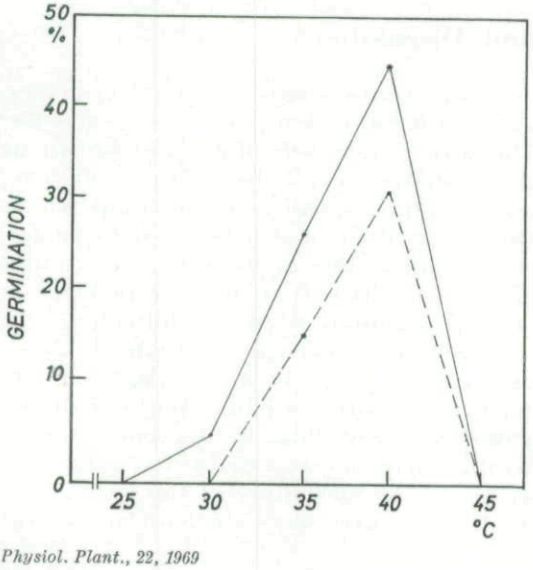

Figure 1. *Temperature effect on spore germination.* (—) Complete medium; (---) potato dextrose agar medium.

Table 2. *Spore density in the medium and mean germination percentage at 40°C.* The spores were presoaked in phosphate buffer for 12 hours.

Spore density	Germination %
900 spores/mm²	94.0
450 „	93.3
200 „	93.5
100 „	90.0
10 „	90.7
10 spores/plate (0.2 spore/mm²)	89.5
1 spore/plate	60.0

(Figure 1). The percentage of spore germination was lower than that in complete medium. A checking of the pH of the media showed that the pH in complete medium and potato dextrose agar medium were 6.8 and 6 respectively. The temperature range for spore germination was rather narrow. A higher temperature was necessary for spore germination than for mycelial growth. The best temperature was 40°C for spore germination and 35°C for mycelial growth. The statistical analysis of data presented in Table 1 indicates that all means of germ tube length at 35°C are greater as compared with those at 40°C, and the differences are all significant at the 1 % level. It was also evident that in our spawn preparation for mushroom beds, mycelium attained the highest growth rate at 35°C. However, germination was strikingly influenced by temperature only as far as unsoaked spores were concerned (Table 1). Germination of spores presoaked in solutions, was not affected since temperature influences the rate of intake of water and of chemical processes involved in germination (10). A relatively mild heat shock necessary to commence the germination process is indicative. The temperature requirement for germination is also concordant to the prediction of Cochrane (6) that the temperature required for spore germination in saprophytic fungi must be higher than 25°C. This temperature is an average requirement for the pathogenic species. However, in some incidents, 25°C also permits germination if the Petri dishes sown with spores are incubated overnight at 40°C and then transferred to 25°C. Germination in these dishes was 23.66 % and in those dishes incubated continuously for two days in 40°C was 73.4 %. This can be explained by the fact that the germination process, when once initiated at a higher temperature, is irreversible and has to go on although the dishes have been brought to a lower temperature which normally does not favour germination.

Soaking of spores. — When the spores were kept in a liquid, either in distilled water or in 0.05 *M* phosphate buffer at pH 8.0 the night before inoculation to the medium, germination increased markedly. Irrespective of the degree of temperature during germination and the value of pH in medium, comparisons of unsoaked spore germination with presoaked ones all exceed the 1 % level of significance (Table 1). It is clear that the spores pretreated either with distilled water or with phosphate buffer give a substantial measure of improving the spore germination, which is probably

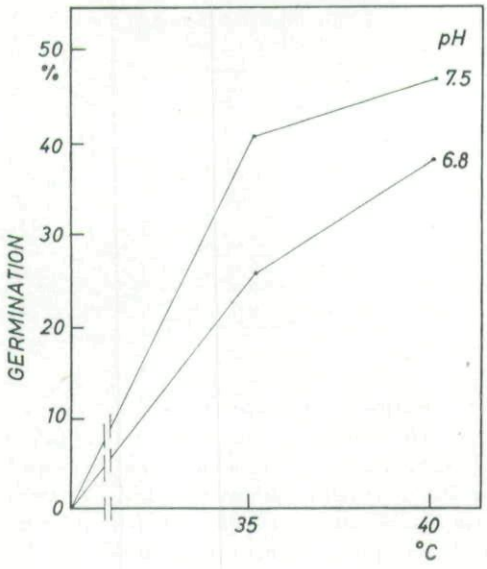

Figure 2. *Effect of pH of medium on spore germination.*

activated by (a) breakage of some permeability barriers in the spores; or (b) washing off of inhibitory substances present in the spores. If a barrier is present, the function of this barrier may depress the permeability rate but not the inhibition of permeability, as a low percentage of germination can still be obtained without soaking. In other fungi, limited evidence has suggested the presence of an inhibitor in spores of *Aspergillus niger* and *Coccomyces hiemalis* at the time of their formation which can be removed by washing (6). The high germination attained by soaking also agrees with the germination conditions in rusts (4) and smut (14) which include presoaking treatment for maximum germination. A dissipation of self inhibitors occurs by this procedure in rust and *Erysiphe* as suggested by Sussman (14).

According to Sussman *et al.* (15), dormant ascospores of *Neurospora* do not permit penetration of larger acid molecules, including phosphate, even after 24 hours because of the unique structure of the spore wall. Basidiospores of *Volvariella,* compared to *Neurospora,* show some difference. The spores of *Neurospora* are completely and evenly surrounded by the wall while in *Volvariella* spores a hilum is present. This thinner area is probably surrounded only by the plasma membrane. However, the structure of the spore wall of *Volvariella* should be studied in detail under electron microscope, like that of *Neurospora,* which has been examined by Lowry and Sussman (11). It is likely that phosphate and other molecules enter the spore of *V. volvacea* through the hilum and activate the germination process.

Effect of spore density. — When spores were first soaked in phosphate buffer, germination increased with increasing spore density. Spores as crowded as 900 spores/mm^2 gave the highest germination. The germination decreased with decrease in number of spores per mm^2 but the difference was not great. A hundred plates each with one single spore, isolated by Raper's

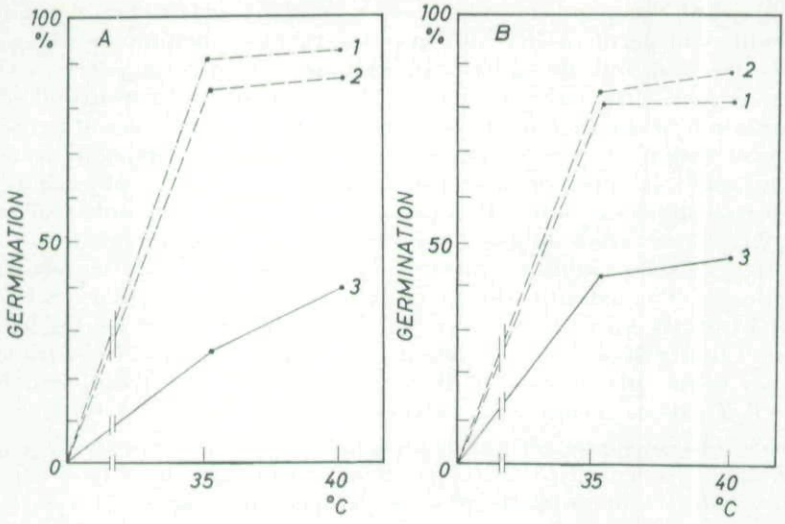

Figure 3. *Effect of presoaking treatment with distilled water and phosphate buffer. A.* On medium with pH = 6.8. *B.* On medium with pH = 7.5. — Between A and B, the differences of phosphate buffer as well as control at both 35° and 40°C are significant at the 1 % level but not in distilled water. Curves: (1) Phosphate buffer; (2) distilled water; (3) control.

spore-cutter were examined. There was a significant decrease in germination (Table 2). This may be due to the increase in carbon dioxide concentration, as CO_2 often accelerates the early growth of fungi (6). The density of spores has some effects on germination as in *Neurospora,* where crowding of spores has reduced the germination rate but not the total germination (6). In *Puccinia graminis* and *P. striiformis,* an increase in spore density will increase the self-inhibition of germination when the spore density is higher than 5400 spores/cm². But in *Psalliota* spp. a few spores germinated will stimulate further germination of other spores (2). Should there be an inhibitor present in the spore, the inhibitor has been removed by washing. The effect of spore density is indicated by the significant difference in germination in plates with individual spores and plates sown collectively with spores.

Effect of pH. — An investigation on the pH of the media was made by varying the pH of complete medium to 7.5, with the controlled dishes at 6.8. The data presented in Table 1 and Figures 2–3 show that: (a) Germination of unsoaked spores was higher in the pH 7.5 dishes, and the differences between two pH values at 35°C as well as at 40°C all exceed 1 % level significance. (b) The mean percentage germination of spores presoaked in distilled water at two different pH values were approximately the same, irrespective of the degrees of temperature. However, after presoaking in phosphate buffer, the germination results were significantly lower in the pH 7.5 dishes at both degrees of temperature. (c) For all soaked spores as well as for unsoaked ones, there was a significant decrease in the germ tube length with increasing pH value at both temperatures. From these observations it

is obvious that the primary effects of increasing pH in the medium comprise changes in permeability and in other surface phenomena. As is known pH influences not only the activity of enzymes but also the entry of vitamins and organic acids (6). Therefore, it may be assumed that for unsoaked spores the stimulation of the higher pH is primarily due to an increased permeability or an acceleration of enzyme activity. For spores presoaked in water there occurs no such stimulation and for spores presoaked in phosphate buffer (pH 8) a retardation is noted. It is probable that the permeability of wall and the activity of enzymes might have changed already during the period of presoaking. Besides, another interesting fact is observed namely that the germ-tubes grow constantly slower in pH 7.5. It may be explained by assuming that high pH inhibits the germ-tube growth as noted by Cochrane (6), who found that a good many basidiomycetes are often unable to grow in culture at an initial pH above 7.0. It is thus necessary to distinguish between the effect of pH on germination of spores and on growth of the germ tubes.

Observations on single germinated hyphae showed that the average increase in hyphal length was 2 to 2.5 μ per hour during the first two hours after the germ vesicle had initiated at room temperature (25°C). Hyphal growth rate may be faster at higher temperature. At the same temperature, different results might be obtained by using different medium as well as different pH value. The basidiospores of this fungus can even germinate in distilled water at suitable temperature (40°C), but germination was relatively low (16 %). The same holds true for phosphate buffer. But the germ tubes were thin and weak compared to those on agar medium, probably due to the liquid environment and lack of nutrition. According to Sussman's definition (14), the maturation of these basidiospores is accomplished before shedding, for spore germination will commence immediately after shedding if the environment is suitable.

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