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EXPERIMENTAL ARTICLES

The Effect of Ecological Factors on Spore Germination and the Viability of the Mycelial Fragments of Microscopic Fungi

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Abstract—Spore germination and the viability of the mycelial fragments of the microscopic fungi *Alternaria alternata, Penicillium spinulosum*, and *Mucor hiemalis* were studied with respect to the action of certain ecological factors: sucrose concentration (0, 0.2, 2, 10, and 100 g/l), temperature (4, 20, 25, and 30°C), pH (3.5, 4.0, 5.0, 6.2, and 7.0), and cadmium concentration (0, 2, 10, and 100 mg/l). The spore germination and the viabilities of different mycelial fragments were found to reach their maxima at different values of the ecological factors studied. This finding suggests that the vegetative and asexual types of reproduction of microscopic fungi may have different ecological optima.

Key words: microscopic fungi, mycelium, spores, viability.

In nature and in laboratory cultures, fungi can grow either from spores or from mycelial fragments. The effect of various ecological factors on fungi is usually studied with respect to their growth from spores [1–4], whereas there are only a few studies dealing with the investigation of fungal growth from mycelial fragments. In our earlier studies, we found that the viability of mycelial fragments is determined by their length, the type of mycelium, the degree of septation, and the method by which these fragments were prepared. We also showed that mycelial fragments whose length is shorter than a *critical fragment size* (CFS) are not viable [5, 6]. The viability of mycelial fragments also depends on the part of hypha from which these fragments were prepared [7]. The ecological aspects of the viability of mycelial fragments have yet to be appropriately studied.

The aim of the present study was to investigate the effect of various ecological factors on the spore germination and on the viability of the mycelial fragments of some microscopic fungi as a function of the fragment length.

MATERIALS AND METHODS

Experiments were carried out with structurally and taxonomically different microscopic fungi *Mucor hiemalis* Wehmer, *Penicillium spinulosum* Thom, and *Alternaria alternata* (Fr. : Fr.) van Keissler [8], which were isolated from soddy podzolic and gray forest soils.

M. hiemalis, which belongs to the class *Zygomycetes*, has coenocytic (i.e., nonseptated) light-colored mycelium and asexually produces sporangiospores 3–5 μm in diameter.

P. spinulosum, which belongs to the class *Deuteromycetes*, has septated light-colored mycelium and asexually produces small phialospores 2.5 μm in diameter.

A. alternata, which belongs to the same class, has dark-colored (due to the presence of melanins in the cell wall) septated mycelium and asexually produces large multicellular porospores 10–25 μm in diameter.

The viability of mycelial fragments was evaluated as a percentage of growing fragments in each of the several groups of mycelial fragments differing in length [5]. To prepare the fragments, fungal mycelia were grown from spore suspensions in liquid Czapek medium at 25°C for three (*M. hiemalis*) or seven (*A. alternata* and *P. spinulosum*) days, i.e., until the spore formation began. The mycelia were fragmented by shaking them at 180 rpm for 10 min [9]. A 0.05-ml drop of a suspension containing from 4.4×10^3 to 8.9×10^3 mycelial fragments per ml was uniformly spread over the surface of a specimen slide overlain by an agar medium layer. This procedure was performed in triplicate. The number of mycelial fragments observed microscopically in each of the length-based groups studied was no less than ten. The observations were performed using a Goryaev chamber.

Spore germination was studied similarly, by determining the percentage of germination of 100 spores on each of the triplicate specimen slides covered with the agar medium layer. Preliminarily, each of the slides was inoculated with a drop of the spore suspension washed off the surface of Czapek agar slants with 10-day-old fungal cultures. The spore suspensions of *M. hiemalis*

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The values of ecological factors used for the estimation of their effect on the germination of spores and the growth of mycelial fragments

Factor	Values investigated
Temperature, °C	4; 20; 25; 30
Sucrose concentration, g/l	0; 0.2; 2; 20; 100
pH	3.5; 4; 5; 6.2; 7
Cadmium concentration, mg/l	0; 2; 10; 100

and *P. spinulosum* contained 10^6 spores/ml and that of *A. alternata* contained 10^5 spores/ml.

The effect of sucrose concentration, temperature, and pH was studied within the range of their values corresponding to the growth limits of the fungi under study [8]. The cadmium concentration range was chosen according to an ecological normative scale of the heavy metal content in acidic and weakly acidic soils [10]. Control growth conditions corresponded to those commonly used for the isolation and identification of soil fungi [9]: growth at 25°C at pH 6.2–6.5 on Czapek agar containing 2% sucrose.

RESULTS AND DISCUSSION

Short mycelial fragments were found to be the most vulnerable to extreme ecological factors. The inability of small fragments to grow and the growth of large fragments led to an increase in the critical fragment size (Fig. 1). For instance, the CFS of the mycelial fragments of *P. spinulosum* increased twofold at high sucrose concentration (100 g/l) and high acidity of the medium (pH 3.5–5.0) and more than tenfold at low temperature (4°C). The CFS of the mycelial fragments of *M. hiemalis* increased twofold at low temperatures. At the same time, the CFS of the mycelial fragments of *A. alternata* remained unchanged under the action of ecological factors. Presumably, the presence of melanins in its cell wall makes this fungus more ecologically resistant in comparison with the other fungi studied [11].

The responses of the fungi to low and high sucrose concentrations were different. The viability of *P. spinulosum* mycelial fragments of all lengths was higher at low than at high sucrose concentrations. At a concentration of 100 g/l, sucrose inhibited the growth of all mycelial fragments of this fungus and completely suppressed the growth of short fragments 30–50 μm in length (Fig. 2). Conversely, the viability of *M. hiemalis* mycelial fragments of all lengths was higher at high than at low sucrose concentrations; this is consistent with the saccharolytic properties of this fungus [8]. The viability of short (20–60 μm in length) mycelial fragments of *A. alternata* increased at high sucrose concentrations, whereas that of long fragments (> 130 μm in length) was independent of the sucrose concentrations used.

The viability of the mycelial fragments of particular fungi was maximal in the temperature intervals close to the temperature optima of these fungi [8]: 25–30°C for *M. hiemalis*, 20–25°C for *P. spinulosum*, and 20–30°C for *A. alternata*. As was shown earlier, the viability of mycelial fragments at low temperatures considerably decreases [6]. In the present study, we found that all the mycelial fragments of *P. spinulosum*, as well as the short fragments (< 140 μm) of *M. hiemalis*, could barely grow at 4°C (Fig. 2), whereas the viability of long fragments of *M. hiemalis* and that of all fragments of *A. alternata* at this temperature decreased by 1.4–2 and 4–6 times, respectively.

The viability of all mycelial fragments of *P. spinulosum* and *M. hiemalis* was maximal at neutral pH values of the medium, which are optimal for the growth of these fungi [8]. At pH 3.5, the viability of all the mycelial fragments of these two fungi decreased (Fig. 2), and short fragments of *P. spinulosum* 30–70 μm in length and of *M. hiemalis* <140 μm in length could not grow at all (Fig. 1). The short mycelial fragments of *A. alternata* 20–60 μm in length grew slightly better at a pH of 6.3–7.0 than at a pH of 3.5–5.0. At the same time, the viability of the long mycelial fragments of *A. alternata* barely depended on pH.

The presence of cadmium ions in the medium, even at low concentrations of 2 and 10 mg/l, adversely influenced the viability of all of the mycelial fragments of *P. spinulosum* (Fig. 2). At the same time, all cadmium concentrations studied increased the viability of all *A. alternata* mycelial fragments by 1.3–1.7 times and that of the short and medium-length (85–200 μm long) mycelial fragments of *M. hiemalis* by about two times. These data are consistent with those available in the literature indicating that low concentrations of some heavy metals ions, cadmium ions in particular, may stimulate the growth of some fungi [12, 13].

Thus, ecological factors may affect the viability of the mycelial fragments (especially short fragments) of microscopic fungi. The mycelial fragments of the melanin-containing fungus *A. alternata* are the most ecologically resistant.

The data presented in this paper indicate that ecological factors exert similar actions on the germination of spores and the growth of mycelial fragments. For instance, at high sucrose concentrations, spore germination and the viability of small fragments of *M. hiemalis* increased, spore germination and the viability of all mycelial fragments of *P. spinulosum* decreased (Fig. 2), and spore germination and the viability of the mycelial fragments of *A. alternata* remained unchanged. At 4°C, the germination of spores and the growth of mycelial fragments of *A. alternata* and *M. hiemalis* slowed down, while the spores and mycelial fragments (except for the longest ones) of *P. spinulosum* witnessed no germination at all (Fig. 2).

At the same time, the effects of pH and cadmium concentration on the germination of spores and on the

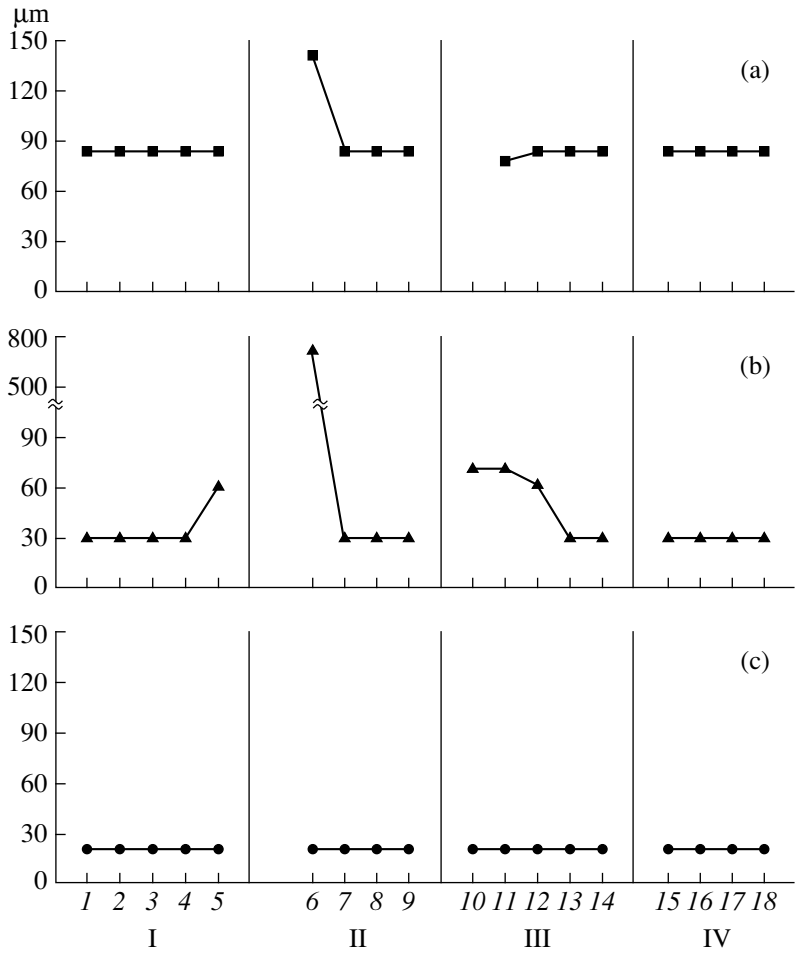

Fig. 1. Changes in the critical fragment size of the mycelial fragments of (a) *M. hiemalis*, (b) *P. spinulosum*, and (c) *A. alternata* incubated in the presence (I) of different sucrose concentrations (g/l): *1*, 0; *2*, 0.2; *3*, 2; *4*, 20; and *5*, 100; (II) at different temperatures (°C): *6*, 4; *7*, 20; *8*, 25; and *9*, 30; (III) at different pH values: *10*, 3.5; *11*, 4.0; *12*, 5.0; *13*, 6.2; and *14*, 7.0; and (IV) in the presence of different cadmium concentrations (mg/l): *15*, 0; *16*, 2; *17*, 10; and *18*, 100.

growth of mycelial fragments were different. For instance, the viability of the mycelial fragments of *P. spinulosum* was maximal at neutral pH values (6.2–7.0) and in the absence of cadmium ions in the medium. Conversely, *P. spinulosum* spores germinated better at acidic pH (4.0–5.0) and in the presence of cadmium ions (Fig. 2). In the case of *A. alternata*, short mycelial fragments grew better at a pH of 6.2–7.0, whereas the number of germinated spores at these pH values decreased by 1.2–1.3 times. When the concentration of cadmium ions rose from 0 to 100 mg/l, the viability of the mycelial fragments of *M. hiemalis* somewhat increased, but the percentage of germinated spores of this fungi decreased by 1.2–1.3 times.

Thus, the viability of mycelial fragments and the germination of spores may reach their highest levels under different ecological conditions.

The fungistatic effect of unfavorable ecological conditions, manifesting itself as a suppression or a delay of spore germination, is a well-known phenomenon [3]. As shown in the present study, the fungistatic effect can also show up as a suppression and a delay of the growth of mycelial fragments. For instance, at 4°C, the mycelial fragments of *A. alternata* and *P. spinulosum* began growing only 48–72 h after the inoculation of the medium [6]. At a cadmium concentration as high as 100 mg/l, the delay in the growth of the mycelial fragments of *P. spinulosum* was 18–24 h.

The fungistatic effect of extreme ecological conditions was more pronounced with respect to fungal spores than with respect to mycelial fragments. For instance, at low temperatures, the mycelial fragments of *M. hiemalis* began growing 24 h after inoculation, whereas its spores began to germinate after 48 h, and the germination rate peaked after 72–96 h. At 30°C in the absence of sucrose, many of the mycelial fragments of *P. spinulosum* (20–40%) began growing 24 h after inoculation; in this case, 35–45% of its spores began germinating after 48 h. Furthermore, cadmium ions caused an 18- to 24-h delay in the growth of the mycelial fragments of *P. spinulosum* only when the concen-

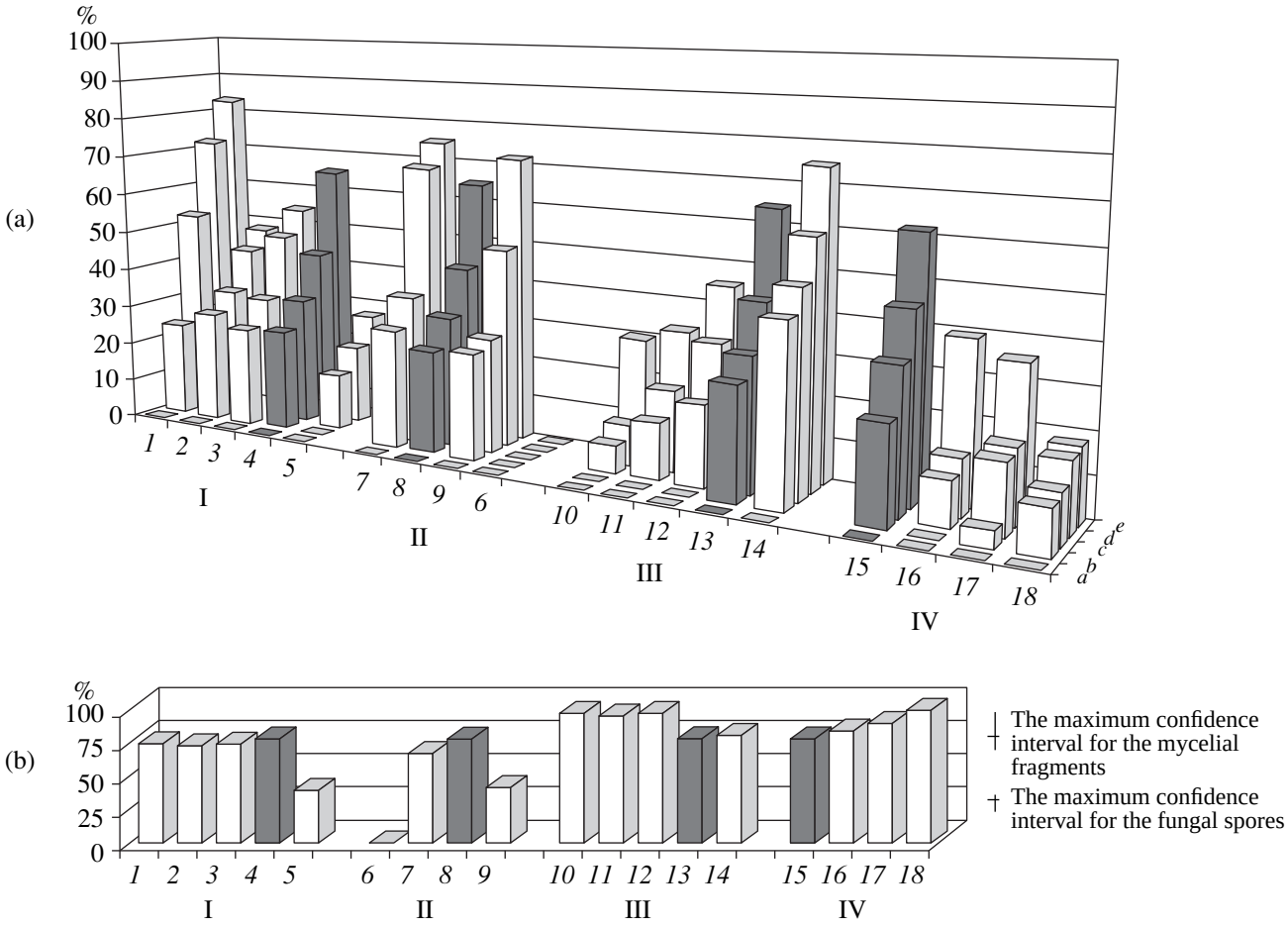

Fig. 2. (a) The viability of the mycelial fragments and (b) the percentage of germinated spores of *P. spinulosum* incubated (I) at different sucrose concentrations (g/l): *1*, 0; *2*, 0.2; *3*, 2; *4*, 20; and *5*, 100; (II) at different temperatures (°C): *6*, 4; *7*, 20; *8*, 25; and *9*, 30; (III) at different pH values: *10*, 3.5; *11*, 4.0; *12*, 5.0; *13*, 6.2; and *14*, 7.0; and (IV) at different cadmium concentrations (mg/l): *15*, 0; *16*, 2; *17*, 10; and *18*, 100. The length of the mycelial fragments (μm): *a*, <30; *b*, 30–50; *c*, 70–90; *d*, 110–130; and *e*, >150. Dark color mark control growth conditions.

tration of these ions was 100 mg/l; the delay in the spore germination was observed at concentrations of cadmium ions as low as 10 or even 2 mg/l.

Thus, the viability of the mycelial fragments of microscopic fungi and their spores depends on the ecological state of the environment. In nature, fungi reproduce either asexually, through spores, or vegetatively, through mycelial fragments. The optimal values of certain ecological factors, such as temperature and organic matter content, are the same for spore germination and the growth of mycelial fragments and fungal colonies [8]. At the same time, the optimum values of other ecological factors, such as pH and cadmium ions (it should be noted that the extreme values of these factors are usually of an anthropogenic nature), may differ for spores and mycelial fragments.

P. spinulosum and *M. hiemalis*, the microscopic fungi that produce light-colored mycelium and small spores, are more susceptible to unfavorable ecological conditions than *A. alternata*, the fungus that produces large spores and the cell-wall melanins.

In general, fungal mycelial fragments are more ecologically resistant than fungal spores, as is shown with reference to *P. spinulosum*, *A. alternata*, and *M. hiemalis* and was shown by other authors with reference to *Penicillium frequentans*, *Aspergillus fumigatus*, *Asp. terreus*, *Verticillium albo-atrum*, *Cladosporium cucumerinum*, and other microscopic fungi [14].

It is common practice to estimate the favorableness of environmental conditions through the growth of fungal colonies from spores [1–3, 9, 11–14]. The experimental data presented in this paper show that the possibility of growth of fungal colonies from mycelial fragments should also be taken into account, especially when evaluating the environmental conditions unfavorable for spore germination. In our opinion, the critical fragment size (CFS) is a convenient parameter that allows for the favorableness of the environment to particular fungal species to be easily appreciated. Under unfavorable conditions for a given species, the CFS of the mycelial fragments of this species increases (and vice versa).

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