Survival and Resistance of *Trichophyton mentagrophytes* Arthrospores

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The effects of several physical and chemical agents on the survival of *Trichophyton mentagrophytes* arthrospores were investigated. Although arthrospores of this dermatophyte were highly resistant to chilling and freezing, they were extremely susceptible to moderate heat (above 50°C) and desiccation. This high susceptibility could be significantly reduced when they were dried in the presence of exogenous proteins. These arthrospores were markedly susceptible to glutaraldehyde. They appeared to be significantly more resistant than their hyphal counterparts to common antimycotics such as clotrimazole, griseofulvin, miconazole nitrate, and nystatin. Clinical and epidemiological implications of these observations are discussed.

*Trichophyton mentagrophytes*, an etiological agent of ringworm in humans and animals, produces abundant arthrospores when parasitizing in keratinized tissues (5, 7). These arthrospores have been suspected of playing an important role in the transmission of dermatomycoses in communal life as well as in the recurrence of certain forms of human dermatomycoses. Despite this epidemiological and possibly clinical importance, the resistance of dermatophyte arthrospores to various physical and chemical agents has not been characterized extensively (1).

During the course of our investigation into physiological factors that affect arthrosporulation of *T. mentagrophytes* (R. G. Emyanitoff, H. J. Blumenthal, and T. Hashimoto, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, 180, p. 168), we developed a method that yielded large quantities of clean individual arthrospores. This allowed us to investigate their survival under various environmental conditions and their resistance to selected physical and chemical agents.

**MATERIALS AND METHODS**

**Microorganism.** *T. mentagrophytes* ATCC 26323 was used throughout this investigation. Stock cultures were maintained at room temperature on Sabouraud-dextrose agar medium (Difco Laboratories), with monthly transfer of the granular-type colonies to prevent the pleomorphic transformation of the fungus.

**Preparation of arthrospores.** Microconidia were produced and purified by the method described earlier (2). Approximately 0.15 to 0.2 ml of microconidial suspension (2 x 10⁶ spores per ml of distilled water) was inoculated over squares (7 by 7 cm) of sterile cellulose dialysis membrane that had been placed on Sabouraud-dextrose agar (Difco) containing 0.1% sodium acetate. The dialysis membrane had been previously boiled in 1% sodium bicarbonate solution for 10 min, rinsed with distilled water, and sterilized by autoclaving. After the inoculated petri plates were incubated at 37°C for 36 h, 4 ml of Sabouraud-dextrose broth (Difco) was added aseptically over the dialysis membrane. By this time, hyphae emerging from microconidia adhered to the membrane and the addition of the broth caused no separation of hyphae from the membrane. The plates were placed in a large glass jar (21 cm in diameter by 25 cm high) and incubated under saturated humidity at 37°C for an additional week. By the end of the incubation period, essentially all hyphae were transformed into arthrospores.

The arthrospores were readily removed from the cellulose membrane by gentle scraping with a spatula. The harvested arthrospores were routinely filtered through 10 layers of cheesecloth to remove residual hyphae or long chains of arthrospores and subsequently washed in ice-cold distilled water at least five times by means of centrifugation (1500 x g, 15 min). After confirming microscopically the absence of hyphal contamination, we dispensed the arthrospores in small, tightly sealed vials and stored them at −20°C until use. Arthrospores remained fully viable under these conditions for as long as 1 year. In most experiments, however, they were used within 1 to 2 weeks after harvesting.

**Effect of temperature.** Arthrospore suspensions (1.5 x 10⁶ cells per ml of distilled water) contained in test tubes were heated at 48, 50, 55, or 60°C or stored at −20, 4, or 10°C for various periods of time; then 0.1 ml of heat-treated or chilled spore suspension was inoculated into 1 ml of Sabouraud-dextrose broth and incubated for 15 h at 37°C on a rotary shaker at 300 rpm. The viability of treated and untreated arthrospores was determined microscopically by counting 200 cells randomly. At the concentration of arthrospores used in the germination system (1.5 x 10⁵ cells...
per ml), it was usually necessary to examine more than 50 randomly selected fields to count 200 arthrospores. Those arthrospores developing distinctive germ tubes (longer than the spore diameter) were considered viable. By the end of 15 h of incubation at 37°C, most germinated arthrospores developed germ tubes as long as 30 to 50 μm.

Effect of ultraviolet light. A 2-ml sample of arthrospore suspension (1.5 × 10⁶ spores per ml of distilled water) was placed in a small petri dish (40 by 10 mm) and irradiated, with the cover removed, at room temperature for a specified time at a distance of 45 cm from the center of a germicidal lamp (Westinghouse Steril lamp 782L-20). Irradiated arthrospores were inoculated into Sabouraud-dextrose broth, and their viability was determined as described above.

Effect of protein on survival of desiccated arthrospores. Since our preliminary experiments revealed that T. mentagrophytes arthrospores were highly susceptible to desiccation, the protective effect of selected proteins on the survival of lyophilized arthrospores was investigated. Arthrospores were initially suspended in 5% (wt/vol) aqueous solutions of powdered skim milk, bovine albumin (crystalline; Sigma Chemical Co.), and gelatin (Difco), and immediately lyophilized. Once lyophilized, they were stored at 25°C for specified periods, and their viability was determined by the method described above.

Effect of antifungal chemicals. Arthrospores suspended in sterile distilled water were exposed to various concentrations of selected antifungal drugs at 25°C. Nystatin (E. R. Squibb and Sons, Inc.), clotrimazole (Delbay Pharmaceuticals, Inc.), miconazole nitrate (Johnson and Johnson Co.), and griseofulvin (Sigma Chemical Co.) were initially dissolved in dimethyl formamide, appropriately diluted with the solvent, and added to spore suspensions so that the final concentration of the solvent would remain 1%. Glutaraldehyde and phenol were obtained from MCB Manufacturing Chemists and used after appropriate dilution in sterile distilled water. Arthrospores exposed to those chemicals under specified conditions were washed in distilled water three times by means of centrifugation (1,500 x g, 15 min) and inoculated into Sabouraud-dextrose broth to determine their viability.

RESULTS

Effect of temperature. Essentially all T. mentagrophytes arthrospores were inactivated within 2 min at 60°C and almost 90% became nonviable within 5 min at 50°C (Fig. 1). Even at 48°C, approximately 50% were killed within 30 min. In contrast to this, the arthrospores were remarkably resistant to chilling and freezing. Those stored in distilled water at 4 or 10°C remained viable (90%) for as long as 2 months. More than 95% of the arthrospores could survive for more than 1 year, and approximately 65% remained viable for 2 years when stored in distilled water at −20°C.

Effect of desiccation. The arthrospores were exceptionally susceptible to desiccation. Both air-dried (25°C) and lyophilized arthrospores lost their viability rapidly during storage, regardless of the storage temperature (Table 1). Their extremely high susceptibility to desiccation was reduced significantly when they were dried in the presence of exogenous proteins (Fig. 2).

Effect of ultraviolet light. The kinetics of killing of arthrospores exposed to ultraviolet light from a common laboratory germicidal lamp is illustrated in Fig. 3. Essentially all were killed within 10 min under our experimental conditions.

Effect of selected disinfectants and chemotherapeutic agents. Resistance to ethanol, phenol, and glutaraldehyde is summarized in Table 2. A remarkably high susceptibility to low concentrations of glutaraldehyde may be worth noting and will be further discussed later. Figure 4 illustrates the survival of arthrospores exposed to various concentrations of antifungal chemotherapeutic agents. It is evident that those ex-

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TABLE 1. Effect of desiccation on viability of T. mentagrophytes arthrospores

<table>
<thead>
<tr>
<th>Desiccation conditions</th>
<th>Storage temp (°C)</th>
<th>% Survival after:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Air dried on cheesecloth, 25°C</td>
<td>4</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>29</td>
</tr>
<tr>
<td>Lyophilized*</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>11</td>
</tr>
</tbody>
</table>

* Arthrospores suspended in sterile distilled water were lyophilized as described in the text.
posed for 24 h to the drugs at concentrations usually lethal to the hyphal growth could remain viable and were able to germinate once the drugs were removed. Under our experimental conditions, it is not certain whether the drugs adsorbed to arthrospores were completely removed during the washing process. In any event, the high degree of resistance of \( T. \) mentagrophytes arthrospores to these chemotherapeutic agents had not been observed previously.

**DISCUSSION**

The present study has provided some new and useful information concerning the resistance of \( T. \) mentagrophytes arthrospores to various physical and chemical agents.

Unlike most bacterial and fungal spores (6), arthrospores (Fig. 1) and microconidia (2) of \( T. \) mentagrophytes are quite susceptible to moderate heat treatment (60°C for 10 min). Arthrospores of \( T. \) rubrum and macroconidia of Microsporum gypseum have been found to be similarly sensitive to moderate heat treatment (unpublished data). These observations suggest that pasteurization or similar heat treatments may destroy most of the dermatophytes and spores they produce and could be used to launder contaminated clothes or to disinfect shower room floors and mats.

Although these arthrospores are highly susceptible to desiccation (Table 1), the presence of exogenous proteins markedly improved the rate of survival under desiccation conditions (Fig. 2). This may imply that dermatophytic arthrospores in fallen hairs and loosened squames from infected individuals are quite able to survive

| TABLE 2. Arthrosporocidal activities of aqueous ethanol, phenol, and glutaraldehyde at 25°C |
|----------------|----------------|----------------|
| Exposure time (h) | % Survival after exposure to: | |
|                 | Ethanol (70%) | Phenol (1%) | Phenol (0.1%) | Glutaraldehyde (0.1%) |
| 0.5             | 0             | 93           | 0             | 97          | 0             | 7             | 0             |
| 1               | 0             | 88           | 0             | 90          | 0             | 0             |
| 3               | 0             | 85           | 0             | 90          | 0             | 0             |

Fig. 2. Protective effect of proteins on survival of lyophilized \( T. \) mentagrophytes arthrospores. Arthrospores were lyophilized in the presence of specified proteins as described in the text and stored at 25°C. Symbols: ▲, powdered skim milk; ×, albumin; ○, gelatin; and ◦, control (no proteins). All points except for day 1 data for gelatin are significantly different (P < 0.01) from those of the controls.

Fig. 3. Effect of ultraviolet light irradiation on viability of \( T. \) mentagrophytes arthrospores.

Fig. 4. Effect of a 24-h exposure (25°C) to various concentrations of antidermatomycotic drugs on survival of \( T. \) mentagrophytes arthrospores. Symbols: ●, clotrimazole; ×, nystatin; ▲, griseofulvin; and □, miconazole nitrate. Arrows indicate minimal inhibitory concentration of each drug for hyphal growth.
under desiccated conditions for some time, and it poses a serious epidemiological problem. To our knowledge, no data are available at this time which deal with the survival of dermatophytic arthrospores in loosened squames from infected patients.

Fungal spores are in general quite resistant to desiccation (6) although certain types of spores are reportedly highly susceptible to dehydration. For example, conidia of Erysiphegraminis polygoni and Oidium heveae, basidiospores of Tilletia tritici, and gemmae of Omphalia flavida were reported to be rapidly inactivated when completely desiccated (6). The exact mechanism whereby certain fungal spores are rapidly killed by desiccation is not known at this time. However, it is likely that the loss of water from vital cellular components may be the primary cause of death. It is probable that the presence of an additional wall layer or spore coat may render certain types of fungal spore more tolerant to desiccation than others by minimizing water loss from the critical core. It is pertinent to recall that the surface of T. mentagrophytes microconidia, which are highly resistant to desiccation (unpublished data), is coated by a proteinaceous rodlet layer (3, 8).

That T. mentagrophytes arthrospores exposed to several antifungal agents at concentrations exceeding the minimal mycelial growth inhibitory level could remain viable (Fig. 4) is believed to have some important clinical and microbiological implications. First, it implies that the administration of these chemotherapeutic agents to dermatomycotic patients whose lesions contain abundant arthrospores (4) may not produce any anticipated therapeutic effects. This could account for the fact that exacerbation or intermittent recrudescence of infections often takes place in such patients when administration of the drugs is discontinued. Second, our data suggest that assessment of antifungal activities of prospective drugs for the treatment of ringworm infections should take into consideration their effect on arthrospores as well as on vegetative hyphae.

Glutaraldehyde may be an effective antidermatophytic agent since, at concentrations as low as 0.01%, it destroys all known forms of the dermatophyte, microconidia, arthrospores, and vegetative hyphae. Glutaraldehyde and factors affecting its efficacy as an antimycotic are now being evaluated in our laboratory.

ACKNOWLEDGMENTS

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LITERATURE CITED