Survival and Resistance of *Trichophyton mentagrophytes* Arthrospores

TADAYO HASHIMOTO* AND HAROLD J. BLUMENTHAL

Department of Microbiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153

Received for publication 26 September 1977

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 antymycotics 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 arthroporulation of *T. mentagrophytes* (R. G. Emyniatoff, 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 × 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 × 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 × 10⁶ cells per ml of distilled water) containing 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 × 10⁶ 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 7821-20). 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 these chemicals under specified conditions were washed in distilled water three times by means of centrifugation (1,500 × 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-
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**

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>% Survival after exposure to:</th>
<th>Ethanol (70%)</th>
<th>Phenol</th>
<th>Glutaraldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1%</td>
<td>1.0%</td>
<td>0.001%</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>93</td>
<td>0</td>
<td>97</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>88</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>85</td>
<td>0</td>
<td>90</td>
</tr>
</tbody>
</table>
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 *Erysiphe graminis polygoni* and *Oidium heveae*, basidiospores of *Til-
letia tritici*, and gemmae of *Omphalina 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* microconi-
dia, which are highly resistant to desiccation
(unpublished data), is coated by a proteinaceous
rodlet layer (3, 8).
That *T. mentagrophytes* arthrospores exposed
to several antimycotics at concentrations ex-
ceeding the minimal mycelial growth inhibitory
level could remain viable (Fig. 4) is believed to
have some important clinical and microbiologi-
cal implications. First, it implies that the admin-
istration of these chemotherapeutic agents to
dermatomycotic patients whose lesions contain
abundant arthrospores (4) may not produce any
anticipated therapeutic effects. This could ac-
count for the fact that exacerbation or intermit-
tent recrudescence of infections often takes place
in such patients when administration of the
drugs is discontinued. Second, our data suggest

that assessment of antidermatophytic activities
of prospective drugs for the treatment of ring-
worm infections should take into consideration
their effect on arthrospores as well as on vege-
tative hyphae.
Glutaraldehyde may be an effective antider-
matophytic 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

This investigation was supported by the Office of Naval
Research.
Excellent technical assistance was provided by Jordan Poll-
lack.

LITERATURE CITED

1977. Development of arthrospores of *Trichophyton
mentagrophytes*. Infect. Immun. 15:968-971.
2. Hashimoto, T., C. D. R. Wu, and H. J. Blumen-
thal. 1972. Characterization of L-leucine-induced germina-
tion of *Trichophyton mentagrophytes* microconidia. J.
Bacteriol. 112:967-976.
3. Hashimoto, T., C. D. Wu-Yuan, and H. J. Blumen-
thal. 1976. Isolation and characterization of the rodlet
layer of *Trichophyton mentagrophytes* microconidial
rospores of *Trichophyton rubrum*. II. Relationship be-
tween the types of eruption and the parasitic forms of
fungi and the pathogenic actinomycetes. The W. B.
Their dormancy and germination, p. 71-74. Harper and
7. Tate, F. 1929. The dermatophytes or ringworm fungi.
and chemistry of microconidial walls of *Trichophyton