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Factors Affecting Germination of *Trichophyton mentagrophytes* Arthrospores

TADAYO HASHIMOTO* AND HAROLD J. BLUMENTHAL

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

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Nutritional and environmental factors affecting germination of *Trichophyton mentagrophytes* arthrospores were investigated. Germination of dormant arthrospores occurred only in rich complex media such as Sabouraud dextrose broth or vitamin-free Casamino Acids. However, once activated, arthrospores were able to germinate under wide ranges of pH (5.5 to 8.0, optimal 6.5) and temperature (20 to 39°C, optimal 37°C) in the presence of certain single amino acids or oligopeptides known to be present in the human cutaneous tissues. Dormant arthrospores could be activated by incubation in distilled water at 25°C for 24 h or by brief exposure to sublethal doses of heat (45°C for 10 to 20 min). Approximately 20% of activated arthrospores underwent spontaneous germination at 37°C during an additional 18 h of incubation in distilled water. All monosaccharides, purines, pyrimidines, and nucleosides tested failed to induce germination of *T. mentagrophytes* arthrospores. Germination rate was affected by the concentration of germination inducers as well as that of arthrospores. The germination process of *T. mentagrophytes* arthrospores was found to be oxygen dependent and was relatively tolerant to NaCl, clotrimazole, cycloheximide, griseofulvin, and tolnaftate.

Trichophyton mentagrophytes is one of the most common fungi causing hunan dermatomycoses, and it produces abundant arthrospores when growing in infected hosts. The arthrospores are considered to be the sole means of reproduction in the parasitic stage in the hairs (12). In fact, arthrospores of dermatophytes are diagnostic in pathological materials (1).

It is generally suspected that arthrospores play vital roles in the transmission and the recurrence of dermatomycoses in humans. Despite their epidemiological and clinical importance, little is known about the resistance and germination requirements of dermatophytic arthrospores. Miyazi and Nishimura (8) reported that arthrosporpes of *T. rubrum* could germinate in brain heart infusion or in Sabouraud dextrose broth at 37°C but not at 27°C. No other specific requirements for germination of dermatophytic arthrospores have been reported.

It has been recently found (R. Emyanitoff, H. J. Blumenthal, and T. Hashimoto, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, I80, p. 168) that abundant arthrospore formation is induced in vitro when the hyphae of *T. mentagrophytes* are incubated at 37°C under slightly reduced oxygen tension, but are not induced by carbon dioxide as previously reported (6). Also, under slightly reduced humidity, arthrospore chains undergo almost complete disarticulation, forming single-celled or short-chained spherical arthrospores (unpublished data).

This paper reports various environmental and nutritional factors that affect the germination of *T. mentagrophytes* arthrospores.

MATERIALS AND METHODS

Microorganism. *T. mentagrophytes* ATCC 26323 was used throughout this investigation.

Maintenance of the fungus. Stock cultures were maintained at room temperature on Sabouraud dextrose agar (4% glucose, 1% neopeptone, and 1.5% agar; Difco Laboratories, Detroit, Mich.).

Preparation of arthrospores. Arthrospores were produced by inoculating approximately 5×10^4 microconidia onto the surface of a Sabouraud dextrose agar plate (9-cm diameter), and the surface of the inoculated plate was covered with 2 ml of sterile distilled water. The inoculated plates were individually sealed with parafilm (Scientific Products, Chicago, Ill.) and incubated at 37°C for 10 days. Under these conditions, this strain of *T. mentagrophytes* was almost completely transformed into arthrospores, and most arthrospore chains underwent disarticulation, forming either spherical or oval-shaped single or short-chained arthrospores. Essentially no microconidia were produced under these conditions. After residual hyphae and long chains of arthrospores were removed by filtration through 10 layers of sterile cheesecloth, arthrospores were washed in cold (4°C) distilled water

at least 10 times by means of centrifugation (10,000 × *g*, 10 min at 4°C). Phase-contrast microscopy of purified arthrospore preparations revealed that there was no contamination with hyphae. The purified arthrospores were usually dispensed in small screw-cap vials (22 by 75 mm), tightly sealed, and kept frozen at −20°C until use.

Germination system. The germination system used in the present investigation was essentially the same as described earlier for germination of microconidia (5). Test tubes, 22 by 75 mm, containing 0.5 ml of *T. mentagrophytes* arthrospore suspension were incubated in the presence of an appropriate concentration of a prospective germination agent on a rotary shaker (model G10, New Brunswick Scientific Co., New Brunswick, N.J.) at 200 rpm under specified conditions. Unless otherwise stated, the germination system contained 0.8×10^5 spores per tube. Samples were removed by appropriate intervals, and percentages of arthrospores developing visible germ tubes were estimated microscopically by counting a total of 100 arthrospores. When multiple samples were removed at one time, glutaraldehyde (25%, MCB Manufacturing Chemists) was added to each tube to a final concentration of 5%, and percentages of germination were determined later as described above.

Effects of temperature and pH on germination. The effect of temperature on arthrospore germination was tested by incubating test tubes containing the mixture of arthrospores and germination inducers in a water bath shaker (New Brunswick model R76) or in a refrigerated water bath (Haake model FK2) preadjusted to a desired temperature. The effect of pH on germination was studied by inoculating spores into germination media preadjusted to desired pH by 0.1 N NaOH or HCl.

Effects of NaCl and antifungal chemicals on germination. Sodium chloride and other antifungal chemicals were incorporated in the germination media at desired concentrations. Those chemicals insoluble in water were initially dissolved in dimethyl formamide and subsequently diluted 100 times with the germination media to obtain desired concentrations.

Effect of oxygen on germination. The requirement of oxygen for the initiation of germination was tested by incubating test tubes containing the mixture of arthrospores and germination inducers in a GasPak jar (BBL, Cockeysville, Md.) at 37°C for 24 h.

Determination of the minimal inhibitory concentration for the hyphal growth of *T. mentagrophytes*. To compare the effect of antifungal chemicals on germination and postgerminative growth, the minimal inhibitory concentration of each drug for the hyphal growth of *T. mentagrophytes* was determined. A series of test tubes, 22 by 75 mm, containing 0.5 ml of Sabouraud dextrose broth and various concentrations of drugs as obtained by the twofold dilution method, were inoculated with approximately 10^5 germinated microconidia and incubated, without agitation, at 30°C for 1 week. Germinated microconidia were obtained as described earlier (3). The minimal concentration of a drug contained in the tube showing neither surface nor submerged growth was macroscopically determined and was taken as the minimal inhibitory concentration for the hyphal growth.

Phase-contrast photomicroscopy. The microscopic appearance of the wet-mounted spores was examined with a phase-contrast microscope by using an oil immersion objective (dark medium, ×100; numerical aperture, 1.25; Nikon). Photomicroscopy was made on panchromatic film (Kodak Plus-X) with a Nikon camera equipped with an automatic exposure system attached to a phase-contrast microscope.

Chemicals. All of the amino acids and peptides used were chromatographically pure and were purchased from Sigma Chemical Co., St. Louis, Mo. Unless specifically mentioned otherwise, all amino acids and dipeptides refer to the L-isomers. Carbohydrates and other biochemicals were from Nutritional Biochemical Co., Cleveland, Ohio. Sabouraud dextrose broth, neopeptone, and vitamin-free Casamino Acids were purchased from Difco. All other chemicals were of reagent grade. Amphotericin B and nystatin were kindly supplied by E. R. Squibb and Sons, Princeton, N.J., clotrimazole by Delbay Pharmaceuticals, Inc., Bloomfield, N.J. and tolnaftate by Schering Laboratories, Kenilworth, N.J. Griseofulvin was purchased from Sigma Chemical Co.

RESULTS

Dark phase-contrast photomicrographs of typical dormant and germinated arthrospores of this fungus are shown in Fig. 1.

Germination of dormant arthrospores. Both freshly harvested arthrospores and frozen stocks of arthrospores of *T. mentagrophytes* (referred to as dormant arthrospores in the subsequent discussion) germinated fairly rapidly, i.e., in less than 7 h, when incubated in rich media such as Sabouraud dextrose broth or in 1% vitamin-free Casamino Acids (Fig. 2A). However, dormant arthrospores germinated very poorly or not at all (<10%) in sodium or potassium buffer (0.05 M, pH 6.5) containing single amino acids or peptides (Table 1 and Fig. 2A). Essentially no germination (<10%) of dormant arthrospores took place during incubation at 37°C for 15 h in distilled water, in tap water, in physiological saline solution (0.85% NaCl), in sodium or potassium phosphate buffer (0.05 M, pH 6.5), or in a basic salt solution of Merz et al. (7) containing 2 mM $MgSO_4$, 0.7 μM $FeCl_3$, and 0.6 μM $MnSO_4$.

Germination of activated arthrospores. When dormant arthrospores were stored in distilled water at 25°C for 24 h (these arthrospores will be referred to as activated arthrospores in subsequent discussion), they became conditioned to germinate either spontaneously in distilled water, phosphate buffer, and basic salt solution, or more effectively in the presence of certain single amino acids and peptides (Fig. 2B and Table 1). Treatment with sublethal doses of heat also appeared to facilitate the activation of dormant arthrospores (Table 2). Activated arthrospores germinating spontaneously in dis-

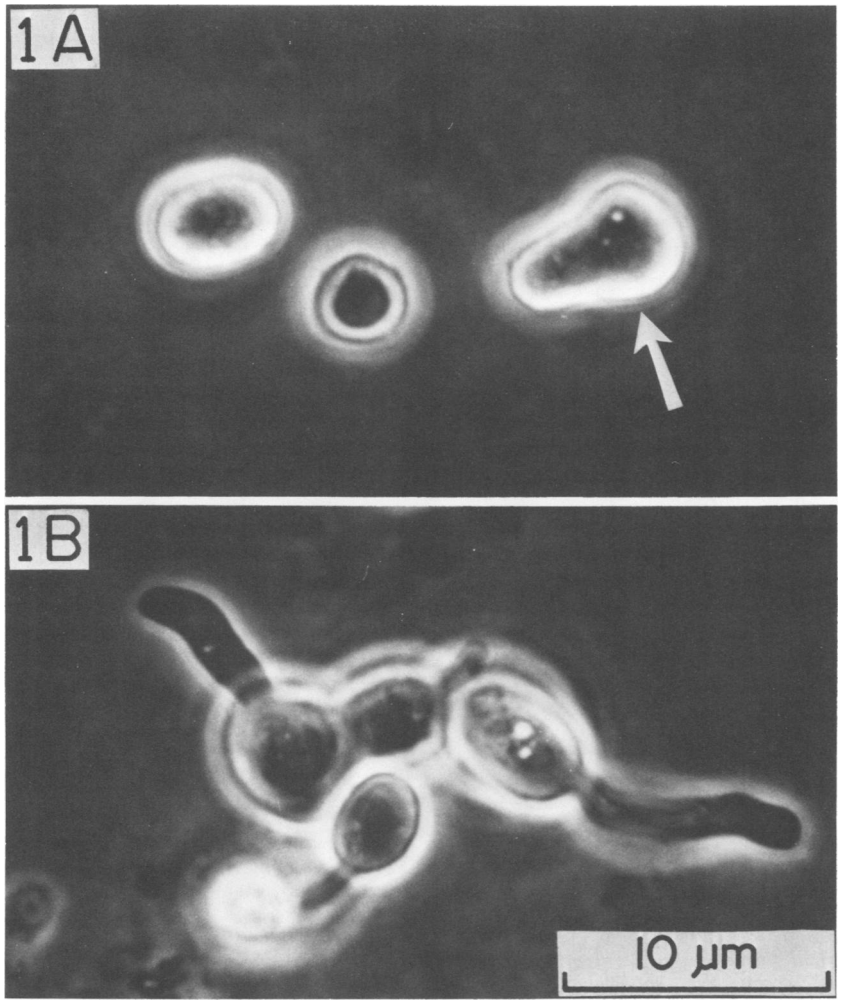

FIG. 1. *Dark phase-contrast micrographs of typical dormant (A) and germinated (B) arthrospores of T. mentagrophytes. Dormant arthrospores were germinated in 1% vitamin-free Casamino Acids (pH 6.5) at 37°C for 7 h. Most arthrospores produced under our experimental conditions were either spherical or oval in shape, but occasionally there were some irregularly shaped spores (arrow in Fig. 1A).*

tilled water, buffer, or saline solution, or in the presence of single amino acids, peptides, or sugars, could develop only short germ tubes (up to several micrometers). Further elongation of germ tubes generally required additional nitrogen and carbon sources.

Effect of chemicals on arthrospore germination. The following individual carbohydrates, organic acids, purines, pyrimidines, and nucleosides failed to induce germination of either dormant or activated *T. mentagrophytes* arthrospores.

Carbohydrates and organic acids. Carbohydrates and organic acids (0.5% in sodium phosphate buffer, 0.05 M, pH 6.5) that failed to induce germination were as follows: D-glucose, D-mannose, L-mannose, D-fructose, D-galactose, D-ribose, L-arabinose, D-xylose, L-xylose, D-rhamnose, D-turanose, D-cellobiose, D-melezitose, D-trehalose, D-melibiose, sucrose, L-sorbose, D-sorbitol, L-arabitol, isoerythritol, dulcitol (galactitol), glycerol, adonitol (ribitol), arabic acid, inulin, salicin, pyruvate (Na), succinate (Na), α-ketoglutarate (Na), *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine, and D-galactosamine hydrochloride.

Purines, pyrimidines, and nucleosides. Non-germination-inducing purines, pyrimidines, and nucleosides (20 mM in 0.1 M sodium phos-

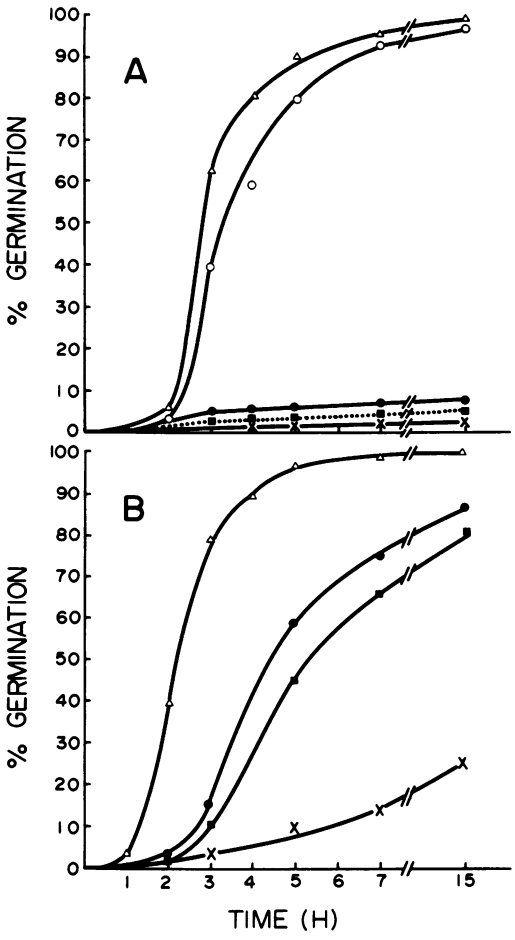

FIG. 2. *Kinetics of germination of dormant (A) and activated (B) arthrospores of T. mentagrophytes in Sabouraud dextrose broth (△), 1% vitamin-free Casamino Acids (○), 10 mM L-leucine (■), 10 mM L-alanine (●), and 0.05 M sodium phosphate buffer, pH 6.5 (×).*

phate buffer, pH 6.5) were as follows: adenine, cytidine sulfate, guanine, uracil, xanthine, hypoxanthine, adenosine, cytosine, guanosine, uridine, 5-methylcytosine-hydrochloride, and inosine.

The effect of concentration of vitamin-free Casamino Acids on germination of activated arthrospores of *T. mentagrophytes* is shown in Table 3. *T. mentagrophytes* arthrospores were able to germinate at concentrations as low as 0.001%. They were able to initiate germination in the presence of single amino acids such as L-alanine, glycine, L-leucine, L-serine, and L-threonine at concentrations as low as 0.02 mM. D-Isomers of certain amino acids could also initiate germination but, in general, less effectivity than L-isomers (Table 4). D-Isomers of the other amino acids listed in Table 1 were either inhibitory to germination or caused germination no more than the controls (15%).

TABLE 1. *Germination of dormant and activated arthrospores of T. mentagrophytes induced by L-amino acids and peptides*[a]

Compound	% Germination[b]	
	Dormant	Activated
Amino acids		
Alanine	<10	85
Arginine hydrochloride	<10	53
Asparagine	<10	23
Aspartic acid	<10	31
Cysteine	<10	25
Cystine	<10	18
Glutamine	<10	30
Glutamic acid	<10	18
Histidine hydrochloride	<10	38
Hydroxyproline	<10	20
Isoleucine	<10	62
Leucine	<10	80
Lysine hydrochloride	<10	14
Methionine	<10	30
Phenylalanine	<10	58
Proline	<10	46
Serine	<10	69
Threonine	<10	68
Tryptophan	<10	52
Tyrosine	<10	71
Valine	<10	68
Glycine	<10	74
Leucine analogs		
Leucyl methyl ester	<10	37
Leucyl ethyl ester	<10	19
N-carbonyl-leucine	<10	21
Peptides		
Leucyl-leucine	<10	57
Leucyl-valine	<10	72
Leucyl-tyrosine	<10	50
Valyl-leucine	<10	13
Valyl-tyrosine	<10	37
Valyl-valine	<10	29
Leucyl-leucyl-leucine	<10	31
Tetra-alanine	<10	61
Penta-alanine	<10	41
Control		
Distilled water	<10	14
Sodium phosphate buffer (0.05 M, pH 6.5)	<10	22
Vitamin-free Casamino Acids	>95	>95

[a] Activation of dormant *T. mentagrophytes* arthrospores was achieved by incubation in sterile distilled water at 25°C for 24 h.

[b] Percentage of germinated arthrospores after incubation of arthrospores at 37°C for 15 h in the presence of 10 mM of each compound in sodium phosphate buffer (0.05 M, pH 6.5), determined as described in the text.

Effect of temperature and pH on arthro-

TABLE 2. *Effect of storage temperature upon subsequent germination of dormant T. mentagrophytes arthrospores*[a]

Treatment	VFCA[b]	% Germination in:				
		L-Alanine	L-Leucine	L-Serine	Glycine	Control[c]
None	>95	<10	<10	<10	<10	<10
4°C, 24 h	>95	<10	<10	<10	<10	<10
25°C, 24 h	>95	85	80	69	74	24
45°C, 10 min	>95	48	60	53	45	18
45°C, 20 min	76	79	71	42	52	16
50°C, 15 min	24	<10	<10	<10	<10	<10

[a] Dormant arthrospores suspended in distilled water were subjected to temperature treatment as specified and subsequently allowed to germinate at 37°C for 15 h in sodium phosphate buffer (0.05 M, pH 6.5) containing 1% vitamin-free Casamino Acids or 10 mM amino acids.

[b] 1% vitamin-free Casamino Acids.

[c] Sodium phosphate buffer (0.05 M, pH 6.5).

TABLE 3. *Effect of concentration of vitamin-free Casamino Acids (VFCA) on germination of T. mentagrophytes arthrospores*

Concn of VFCA (%)	% Germination[a]
1.0	>95
0.5	>95
0.1	93
0.05	89
0.01	76
0.005	76
0.001	73
0.0	21

[a] Percentage of germinated arthrospores after incubation at 37°C for 15 h in the specified concentration of VFCA in sodium phosphate buffer (0.05 M, pH 6.5), determined as described in the text.

TABLE 4. *Germination of activated arthrospores of T. mentagrophytes induced by D-amino acids*

D-Amino acids	% Germination[a]
Alanine	26
Asparagine	28
Isoleucine	31
Leucine	33
Serine	31
Threonine	32
Sodium phosphate buffer (0.05 M, pH 6.5)	15
Distilled water	15
1% vitamin-free Casamino Acids	>95

[a] Percentage of germinated arthrospores after incubation at 37°C for 15 h in the presence of 10 mM of each compound in sodium phosphate buffer (0.05 M, pH 6.5), determined as described in the text.

spore germination. Germination of *T. mentagrophytes* arthrospores could take place in Sabouraud dextrose broth under wide ranges of temperature (Fig. 3) and pH (Fig. 4). However, the optimal temperature for germination in vitamin-free Casamino Acids or in L-leucine was found to be 37°C. Similarly, the optimal pH for germination in vitamin-free Casamino Acids or in L-leucine was 6.5.

Effect of spore concentration on arthrospore germination. Apparently, the concentration of arthrospores critically affected the rate of germination induced by amino acids. Essentially no germination took place, even under optimal conditions, when the concentration of arthrospores reached 10^7 cells per ml (Table 5). When properly diluted, all these arthrospores underwent germination normally.

Requirement of oxygen for arthrospore germination. Oxygen appeared to be essential for the initiation of germination of arthrospores. No germination occurred when activated arthrospores were incubated in Sabouraud dextrose broth or in 1% vitamin-free Casamino Acids under strict anaerobic conditions.

Effect of NaCl and certain antidermatophytic compounds on arthrospore germination. Germination of *T. mentagrophytes* arthrospores in vitamin-free Casamino Acids was only slightly inhibited by high concentrations (5%) of NaCl (Table 6). The effects of various antifungal chemicals on germination of *T. mentagrophytes* arthrospores are shown in Table 7. Apparently the germination process of *T. mentagrophytes* arthrospores was less susceptible to certain antifungal agents than was the growth of the hyphal form.

DISCUSSION

The present investigation was greatly facilitated by the availability of clean, single-celled arthrospore preparations and has added some new and significant information as to the germination requirements of *T. mentagrophytes* arthrospores.

It is evident from the data presented (Fig. 2, Tables 1 and 2) that dormant arthrospores of *T. mentagrophytes,* like many other microbial spores (11), are rendered more readily respon-

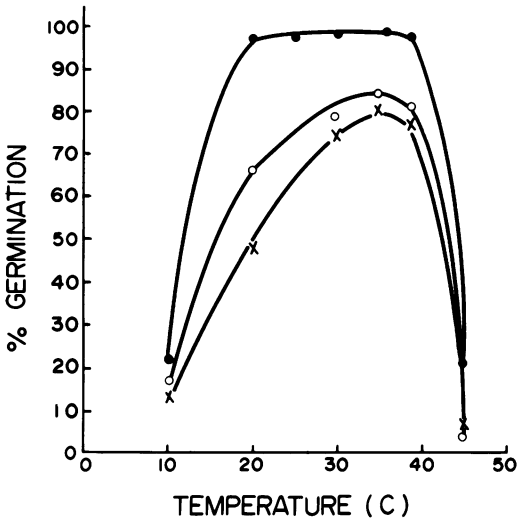

FIG. 3. *Effect of temperature on germination of activated T. mentagrophytes arthrospores. Arthrospores were germinated for 15 h in Sabouraud dextrose broth (●), 1% vitamin-free Casamino Acids (○), and 10 mM L-leucine (×). pH of the media was adjusted to 6.5.*

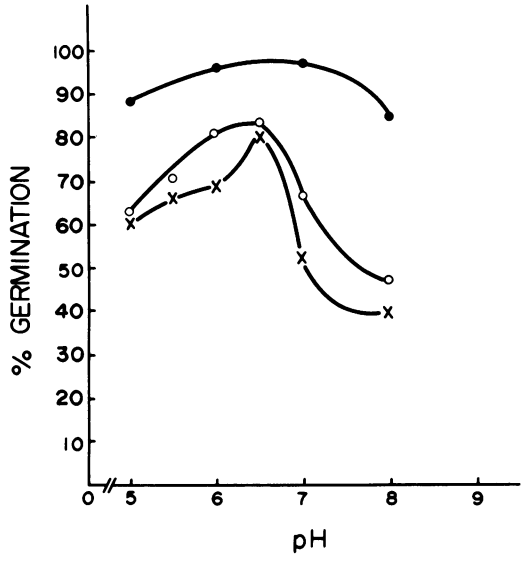

FIG. 4. *Effect of pH on germination of activated T. mentagrophytes arthrospores. Arthrospores were germinated for 15 h in Sabouraud dextrose broth (●), 1% vitamin-free Casamino Acids (○), or 10 mM L-leucine (×), adjusted to the indicated pH.*

sive to germination inducers when properly activated. Under our experimental conditions, exposure to distilled water at 25°C for 24 h or sublethal heating at 45°C for 10 to 20 min was sufficient to condition the majority of dormant

TABLE 5. *Effect of cell concentration on germination of activated T. mentagrophytes arthrospores*

Spore concn cells/ml	% Germination[a]		
	VFCA[b]	L-Alanine	L-Leucine
0.8×10^5	>95	85	80
3.4×10^5	81	66	64
8.5×10^5	84	8	31
1.7×10^6	39	3	5
3.4×10^6	23	1	1
1.0×10^7	0	0	0

[a] Arthrospores were activated by incubating in sterile distilled water at 25°C for 24 h and were then germinated at 37°C for 15 h in sodium phosphate buffer (0.05 M, pH 6.5) containing 1% vitamin-free Casamino Acids or 10 mM amino acids.
[b] 1% vitamin-free Casamino Acids.

TABLE 6. *Effect of NaCl on germination of T. mentagrophytes arthrospores*

Concn of NaCl (%)	% Germination[a]
0	>95
0.5	>95
1.0	>95
2.0	>95
5.0	58
10.0	9

[a] Percentage of germinated arthrospores after incubation at 37°C for 15 h in the presence of 1% vitamin-free Casamino Acids supplemented with the specified concentration of NaCl in sodium phosphate buffer (0.05 M, pH 6.5), determined as described in the text.

arthrospores to germinate in the presence of limited exogenous nutrients (Fig. 2, Table 1 and 2). Interestingly, approximately 20% of the activated arthrospore population underwent germination even in the complete absence of exogenous nutrients when sufficient temperature and humidity were provided (Table 1). It may be significant that most of those amino acids capable of inducing germination of activated arthrospores happened to be the predominant free amino acids normally present in human cutaneous tissues (2, 10). These observations, combined with the optimal temperature (30 to 37°C) and pH (6.5) for amino acid-induced germination of *T. mentagrophytes* arthrospores (Fig. 3 and 4), may account for the common experience that certain forms of dermatomycoses frequently show spontaneous recurrence during humid seasons. Arthrospores remaining viable in lesions may be insidiously activated as the result of prolonged exposure to water or increased humidity in the skin, and germination may be initiated either spontaneously or by a trace amount of certain amino acids or peptides produced by

TABLE 7. *Effects of antifungal compounds on germination of T. mentagrophytes arthrospores*

Concn of compound[a] (μg/ml)	% Germination[b]					
	Amphotericin B (5 μg/ml)	Clotrimazole (0.5 μg/ml)	Cycloheximide (100 μg/ml)	Griseofulvin (2.5 μg/ml)	Nystatin (5 μg/ml)	Tolnaftate (2.5 μg/ml)
Control	>95	>95	>95	>95	>95	>95
0.01	>95	>95	>95	>95	>95	>95
0.1	>95	>95	>95	>95	90	>95
0.5	10	>95	>95	>95	88	>95
1.0	2	>95	>95	>95	47	>95
5.0	0	85	>95	>95	8	>95
10	0	72	>95	>95	8	>95
25	0	56	>95	>95	6	86

[a] These compounds are initially dissolved in dimethyl formamide and subsequently diluted 100 times to specified concentrations with 1% vitamin-free Casamino Acids (VFCA). Control contained 1% dimethyl formamide in 1% VFCA.

[b] Percentage of germinated arthrospores after incubation at 37°C for 15 h in the presence of 1% VFCA supplemented with specified final concentrations of antifungal compounds in sodium phosphate buffer (0.05 M, pH 6.5), determined as described in the text. Figures in the parentheses represent the minimal inhibitory concentrations for hyphal growth of *T. mentagrophytes* as determined by the method described in the text.

skin proteases (3, 4). It has been noted (unpublished observation) that *T. mentagrophytes* arthrospores, inoculated on isolated human stratum corneum, can germinate and transform into hyphae as long as sufficient humidity is provided. These observations, together with the data presented in this paper, strongly suggest that arthrospores of dermatophytes are potential sources of reinfection or exacerbation in dermatomycotic patients. The facts that arthrospores are formed abundantly in infected tissues and their germination requirements are relatively nonstringent may also suggest that they play a vital role in the transmission of infections through public facilities such as locker and shower room floors in communal life.

The ability of *T. mentagrophytes* arthrospores to germinate either in the presence of very limited nutrients or in the total absence of exogenous nutrients suggests that the arthrospores contain endogenous nitrogen and carbon sources essential for the initial germ tube formation. Schmit and Brody (9) found all of the common amino acids, except proline, methionine, and cystine, in the free amino acid pool of spore extracts of *Neurospora crassa*.

The observation that the germination process was not significantly affected by the minimal growth-inhibitory doses of clotrimazole, griseofulvin, and tolnaftate (Table 7) implies that either arthrospores are less permeable to these drugs or reactions susceptible to these drugs are absent in the germinating arthrospores. It is also probable that certain compounds essential for germination are preformed during arthrosporogenesis and stored intracellularly, although the biosynthesis of such compounds is affected by these agents.

The amino acids capable of inducing germination of *T. mentagrophytes* arthrospores (Table 1) and microconidia (2) are generally similar but not identical. L-Leucine, L-isoleucine, L-alanine, L-valine, and glycine are effective germination inducers for both arthrospores and microconidia. L-Serine, L-threonine, and L-tyrosine are effective germination inducers only for arthrospores and not for microconidia. Conversely, L-methionine is a relatively good germination inducer for microconidia, but not for arthrospores.

At present, the mechanism of arthrospore germination by single amino acids or by peptides is not known. It also remains to be determined whether the germination of arthrospores induced by certain peptides (Table 1) was triggered directly by peptides themselves or was due to the indirect action of free amino acids deriving from the enzymatic cleavage of peptides by spore peptidases.

The total or partial inhibition of germination in heavily populated spore suspensions (Table 5) has been known for many types of microbial spores (11). Since spores were able to resume germination upon dilution, it is likely that this inhibition is due to the competition of reactive sites of spores for available germination inducers, although the presence of germination inhibitors in these spores cannot be totally excluded. The presence of a specific germination inhibitor in certain fungal spores has been reported (11).

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LITERATURE CITED

1. **Ajello, L., and A. A. Padhye.** 1974. Dermatophytes and the agents of superficial mycoses, p. 469-481. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D. C.
2. **Burke, R. C., T. H. Lee, and V. Buettner-Janusch.** 1966. Free amino acids and water soluble peptides in stratum corneum and skin surface film in human beings. Yale J. Biol. Med. **38**:355-373.
3. **Fraki, J. E.** 1976. Human skin proteases. Separation and characterization of two acid proteases resembling cathepsin B1 and cathepsin D and of an inhibitor of cathepsin B1. Arch. Dematol. Res. **255**:317-330.
4. **Fraki, J. E., and V. K. Hopsu-Havu.** 1975. Human skin proteases. Separation and characterization of two alkaline proteases, one splitting trypsin and the other chymotrypsin substrates. Arch. Dermatol. Res. **253**:261-276.
5. **Hashimoto, T., C. D. R. Wu, and H. J. Blumenthal.** 1972. Characterization of L-leucine-induced germination of *Trichophyton mentagrophytes* microconidia. J. Bacteriol. **112**:967-976.
6. **King, R. D., C. L. Dillavou, J. H. Greenberg, J. C. Jeppsen, and J. S. Jaegar.** 1976. Identification of carbon dioxide as a dermatophytic inhibitory factor produced by *Candida albicans*. Can. J. Microbiol. **22**:1720-1727.
7. **Merz, W. G., K. O. Lloyd, and M. Silva-Hunter.** 1972. Characterization of *Trichophyton mentagrophytes* and its diffusible pigment. Sabouraudia **10**:86-93.
8. **Miyazi, M., and K. Nishimura.** 1971. Studies on arthrospores of *Trichophyton rubrum*. Jpn. J. Med. Mycol. **12**:18-23.
9. **Schmit, J. C., and S. Brody.** 1975. *Neurospora crassa* conidial germination: role of endogenous amino acid pools. J. Bacteriol. **124**:232-242.
10. **Schwarz, E.** 1971. Freie aminosäuren und verwandet Verbindugen in der abschabbaren Hornschicht unbefallener Haut von mikrobiellen Ekzematikern und Hautgesunden. Arch. Dermatol. Forsch. **242**:87-96.
11. **Sussman, A. S., and H. O. Halvorson.** 1966. Spores: their dormancy and germination. Harper & Row, Publishers, New York.
12. **Tate, P.** 1929. The dermatophytes or ringworm fungi. Biol. Rev. Biol. Proc. Cambridge Philos. Soc. **4**:41-75.