Macroconidial Development and Germination in *Trichophyton mentagrophytes*

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Trichophyton Mentagrophytes was investigated for macroconidial development with particular emphasis on the conidial ageing by light and scanning electron microscopy. Macroconidial germination was also studied under various conditions. Sabouraud glucose agar supplemented with 3% NaCl was used to enhance production of macroconidia. After a long-term cultivation macroconidial compartments changed to spherical thick-walled structure. Some 12-month-old macroconidia were still capable of germination. A wide range of temperature (15–37°C), and inoculum of less than 1×10^5 conidia per ml of rich media were appropriate for macroconidial germination. The germination process of macroconidia was highly tolerant to NaCl. A small fraction of the conidia were able to germinate even in distilled water without activation. Effect of freeze-thaw or ultraviolet irradiation on macroconidial germination was determined. *J Invest Dermatol 90:165–169, 1988*

The morphology of macroconidia is of prime importance in the classification and identification of dermatophytes [1; for a review, see 2]. In addition, macroconidia of dermatophytes, like their microconidia, are at least under some conditions considered to be infectious. In spite of such importance, the biologic features of dermatophyte macroconidia are relatively ill-defined. This is especially true for *Trichophyton mentagrophytes,* the most common cause of human skin infection, probably because this fungus is a poor producer of macroconidia. Only fragmentary observations have been made on the ontogeny of macroconidia in this species [3,4], and nothing is known about its germination processes.

During the course of investigation of macroconidial production in *T mentagrophytes,* we learned that Sabouraud glucose agar supplemented with NaCl formulated by Kane and Fischer [5] facilitated abundant macroconidia formation. Using this medium, we studied the process of macroconidial development and ageing in *T mentagrophytes* with a light and a scanning electron microscope. We also examined various environmental and nutritional factors for possible effects on the macroconidial germination and discussed their biological and clinical implications including some comparisons with the reported properties of arthroconidia of *T mentagrophytes.*

Manuscript received December 9, 1986; accepted for publication April 6, 1987.

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Abbreviations:

SEM: scanning electron microscopy

UV: ultraviolet

MATERIALS AND METHODS

Fungus and Culture Media The strain of *T mentagrophytes* used in this study, MTU 19002, was provided by Dr. K. Iwata, University of Tokyo, Tokyo. Stock cultures were maintained at room temperature on Sabouraud glucose agar which contained 10 g polypeptone (Daigo Eiyo, Osaka, Japan), 20 g glucose, 15 g agar per liter. Sabouraud glucose agar supplemented with NaCl at 30 g per liter was used for production of macroconidia. Sabouraud glucose broth was for the germination of macroconidia.

Production of Microconidia and Macroconidia Microconidia were obtained by growing the fungus on a Sabouraud glucose agar plate at 25°C for two weeks, harvested by washing the surface of the plate with sterile distilled water followed by centrifugation, washed twice with sterile distilled water, and suspended in sterile saline. Macroconidia were produced by inoculating microconidia $1-5 \times 10^4$ thus prepared onto the surface of a Sabouraud glucose NaCl agar plate and incubating it at 25°C. For large scale preparation of macroconidia, those produced on a Sabouraud glucose NaCl agar slant (150 ml) in a Roux bottle was purified by a modification of the method of Leighton and Stock [6]. Conidia were released from the surface of the mycelial mat by vigorous agitation of the bottle for 30 s with 15 ml of saline containing 0.5% Tween 80 and 1.0% methanol. The resulting suspension was filtered twice through two layers of sterile cottoncloth (42 strings/inch). The conidial suspension was centrifuged at $600 \times g$ for 120 s and the pellet was resuspended in saline followed by centrifugation at $600 \times g$ for 25 s. The latter step was repeated three times.

Light and Scanning Electron Microscopy The slide-culture technique was employed for light microscopy. The cultures were observed directly under a microscope at a magnification of 400×. Samples were prepared for scanning electron microscopy (SEM), using a modification of the method of Akin and Michaels [7]. Colonies grown on Sabouraud glucose NaCl agar in 25-mm diameter Petri dishes were fixed with OsO_4 vapor for 18 h at room temperature in a sealed container. The specimens were then coated with gold-palladium and observed in a JSM-2 scanning electron microscope (JEOL, Tokyo, Japan) with acceleration voltage of 9 kV.

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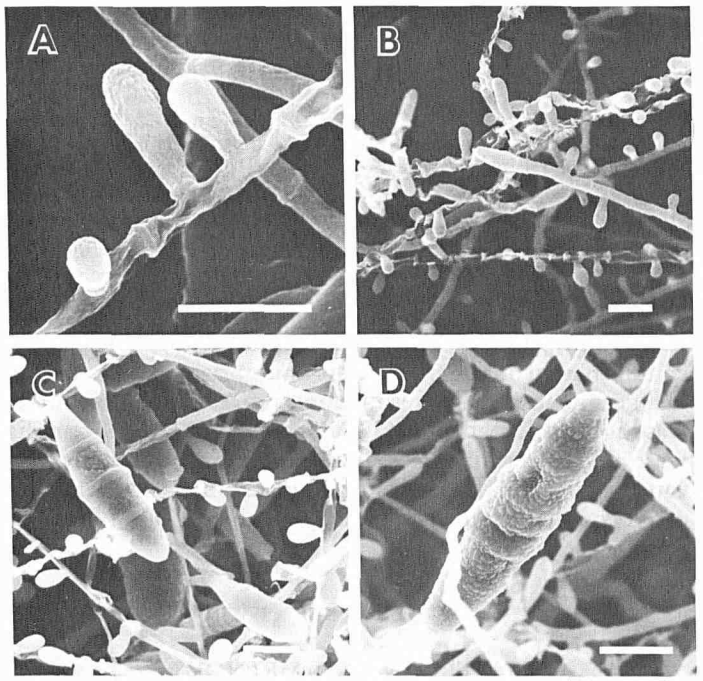

Figure 1. Scanning electron micrographs of macroconidial development. *T mentagrophytes* was grown on Sabouraud glucose agar containing 3% NaCl. A, 3-day incubation. Young macroconidia developing on the side of a hypha. B, 5-day incubation. A macroconidium has appeared at the extremity of a hypha. Many microconidia are also seen. C, 1-week incubation. Matured macroconidia. D, two-week incubation. A macroconidium with coarse surface. Bar, 5 μm.

Germination of Macroconidia Germination experiments were carried out by adding 1×10^4 macroconidia per ml to Sabouraud glucose broth. After 48-h incubation at 25°C, the number of conidia with germ tubes was scored with a microscope. As a criterion, a macroconidium with at least one germinated compartment was considered to have germinating ability. Effect of freezing and thawing on macroconidial survival was determined after cycles of freezing at −70°C and thawing at 37°C. Ultraviolet (UV) sensitivities

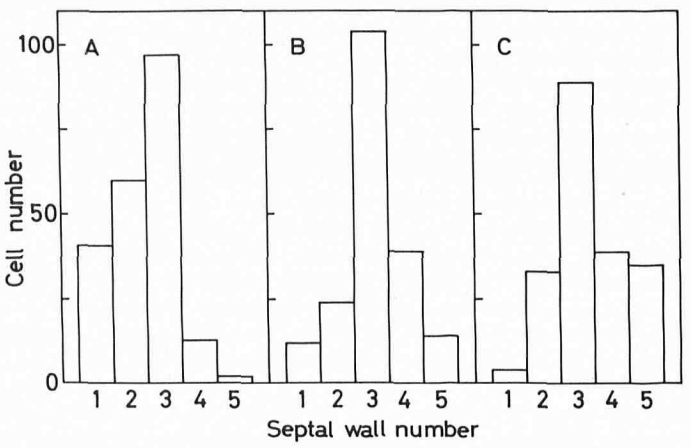

Figure 2. The number of septal walls of macroconidia as a function of incubation period. The fungus was grown on Sabouraud glucose NaCl agar at 25°C. The number of macroconidial septal walls obtained from 7-day-culture [*A*], 14-day-culture [*B*] and 21-day-culture [*C*] were counted under a microscope.

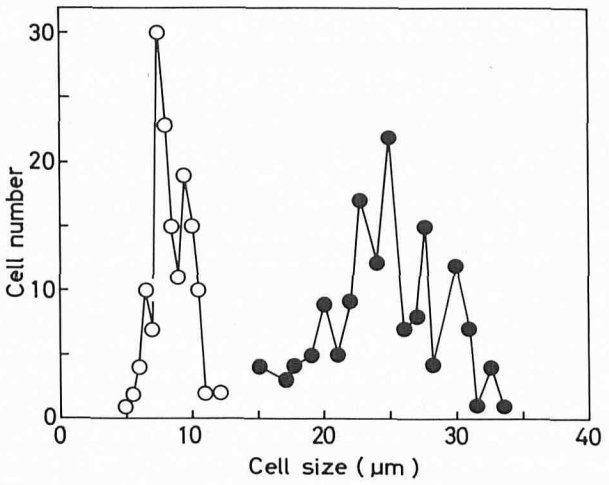

Figure 3. Distribution of size of macroconidia. *T mentagrophytes* macroconidia obtained from two-week culture on Sabouraud glucose NaCl agar at 25°C were measured cell width (empty circles) and cell length (solid circles) with a micrometer under a microscope.

of microconidia and macroconidia were tested by exposure of conidia under a 15-W germicidal lamp at a distance of 45 cm.

RESULTS

Development and Structure of Macroconidia The process of development of *T mentagrophytes* macroconidia on Sabouraud glucose NaCl agar as observed by SEM is shown in Fig 1. In 3- to 5-day cultures, young macroconidia developing as short branches on the side of a hypha, or as a swelling at the extremity of a hypha were seen (Fig *1A, B*). After one week of incubation macroconidia were septate with three or more compartments, spindle shaped and smooth surfaced (Fig 1*C*). Coarse-surfaced macroconidia were also often seen (Fig. 1*D*).

Septal walls were seen as protruded circles at irregular intervals (Fig. 1*C,D*). Septation seems to have started at an early stage of macroconidial production and as shown in Fig 2, the average number of septal walls per conidium increased with time of incubation. Macroconidia possessing three septal walls were most common and those having as many as five septal walls were observed (Fig 2). The

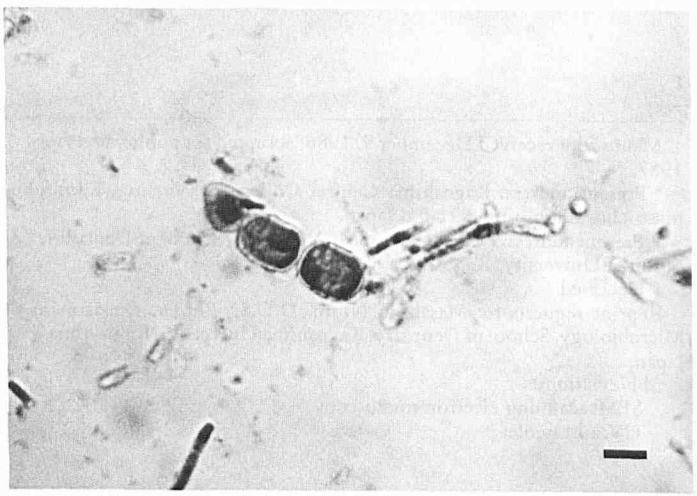

Figure 4. Light micrograph of one-month-old macroconidia with spherical compartments. *T mentagrophytes* macroconidia were obtained from the culture grown on Sabouraud glucose NaCl agar at 25°C. Bar, 5μm.

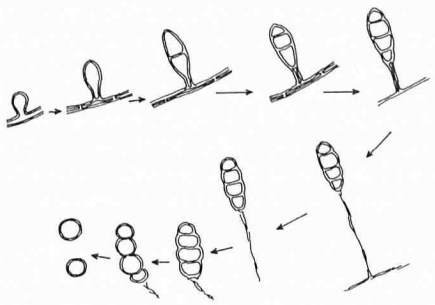

Figure 5. Diagrammatic representation of the morphogenesis of macroconidia. *T mentagrophytes* was grown on Sabouraud glucose NaCl agar at 25°C.

size distribution of macroconidia is shown in Fig 3, the average size being 7.5 μm in width and 25 μm in length.

After about two weeks incubation, the macroconidia-rich layer was covered by newly formed mycelia, so that the conidia could not be seen from outside of the mycelial mat. These secondary hyphae did not produce any macroconidia. In one-month-old cultures, macroconidial septal walls were seen to have split centripetally and the compartments become spherical as shown by light microscopy (Fig 4). Further incubation resulted in separation of macroconidial compartments, which looked like thick-walled chlamydospores as observed by SEM as well as by light microscopy (data not shown). From these results, the morphogenesis of macroconidia in *T mentagrophytes* could be schematized as illustrated as Fig 5.

Macroconidial Germination Mature macroconidia prepared from two-week cultures were examined for the effects of various conditions on germination. As shown in Fig 6, the most suitable temperature range giving 100% germination in Sabouraud glucose broth was 18–25°C. Seventy per cent of macroconidia germinated at 15, 30, or 37°C. Germination did not occur at temperatures lower than 7°C or higher than 42°C. Inoculum size also affected germination as depicted in Table I: inoculum of 1×10^3 cells per ml was

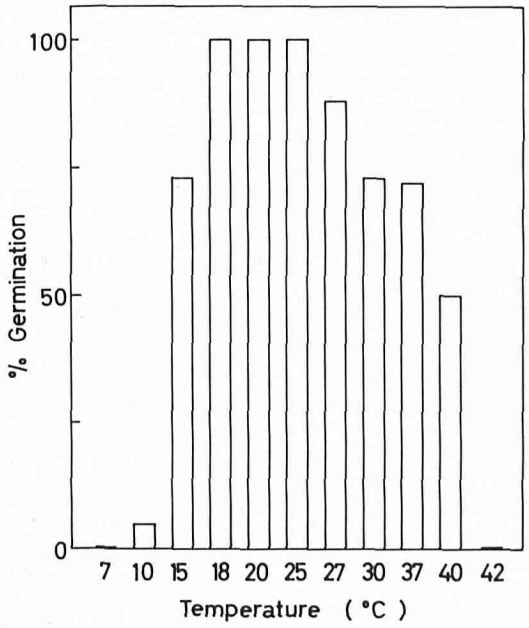

Figure 6. Effect of temperature on macroconidial germination. *T mentagrophytes* macroconidia obtained from two-week cultures on Sabouraud glucose NaCl agar were determined for germination by incubation in Sabouraud glucose broth for 48 h at the test temperature.

Table I. Effect of Inoculum Size on Germination of Macroconidia of *T mentagrophytes*

Inoculum Size (conidia per ml)	Germination (%)	
	Conidium[a]	Compartment[b]
1×10^3	100	100
1×10^4	100	87.5
1×10^5	45	27.9
1×10^6	0	0

Macroconidia obtained from a two-week culture on Sabouraud glucose NaCl agar were inoculated at the indicated cell density into Sabouraud glucose broth. They were incubated at 25°C for 48 h to determine germination.

[a] Number of conidia with germ tubes × 100/number of conidia scored.

[b] Number of compartments with germ tubes × 100/number of compartments scored.

most suitable, i.e., all macroconidia germinated from every compartment. At 1×10^6 cells per ml germination did not take place at all. Some macroconidia germinated even in distilled water. More than 60% of the conidia were able to germinate in the medium containing 10% NaCl (Table II).

The relation between conidial age and germinating potential was studied (Fig 7). Macroconidia obtained from 2-month-old or younger cultures germinated well, but the germination rate decreased when incubation period was longer than 4 months. Of special interest was the observation that nearly ten per cent of one-year-old macroconidia retained the ability to germinate.

The effects of freeze-thaw and UV irradiation on the viability were determined with macroconidia obtained from two-week cultures. Eighty per cent of macroconidia survived one cycle of freezing and thawing; complete loss of germinating ability was effected by 6 cycles. There was no discernible difference in UV sensitivity between macroconidia and microconidia (Fig 8).

DISCUSSION

We confirmed the observation by Kane and Fischer [5] that the addition of NaCl at 30 g per liter to Sabouraud glucose agar enhanced the production of macroconidia in *T mentagrophytes,* which enabled us to study various aspects of the conidia. The reason of the promoting effect of NaCl on the macroconidial production is entirely not known, however. To our knowledge, this is the first report to deal with the biologic features of the macroconidia of this important dermatophyte.

The macroconidia of *T mentagrophytes* have been known as club-like, thin-walled, and smooth-surfaced structures as observed by light microscopy. In the present study, coarse-surfaced macroconidia were also observed among typically smooth-surfaced ones. These atypical macroconidia looked somewhat like those of the geuns *Microsporum,* but were devoid of the projections variously named as echinulation [1], verrucose [8], or polyp [7] characteristic of the *Microsporum* macroconidia. Since the coarse-surface morphol-

Table II. Germinating Ability of *T mentagrophytes* Macroconidia in Various Media

Test Medium	Germination (%)
Sabouraud glucose broth	100
Sabouraud glucose broth plus 1% yeast extract	100
Sabouraud glucose broth plus 3% NaCl	72
Sabouraud glucose broth plus 5% NaCl	71
Sabouraud glucose broth plus 10% NaCl	63
Physiological saline	24
Distilled water	18

Macroconidia were obtained from a two-week culture on Sabouraud glucose NaCl agar and were determined for germination by incubating at 25°C for 48 h in each of the test media.

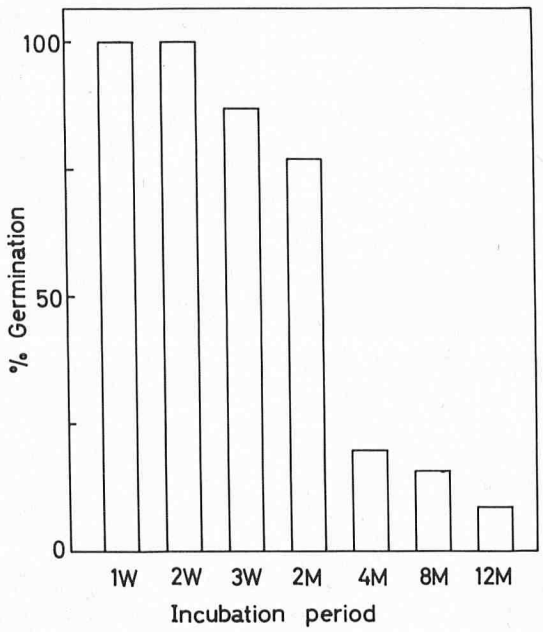

Figure 7. The relation between conidial age and germinating potential of macroconidia. *T mentagrophytes* macroconidia obtained from Sabouraud glucose NaCl agar were determined for germination by incubation in Sabouraud glucose broth for 48 h at 25°C.

ogy was also seen among macroconidia occasionally produced on Sabouraud glucose agar without NaCl, it was considered not to be an artifact caused by the high concentration of NaCl.

Some aspects of the developmental process of *T mentagrophytes* macroconidia were also presented; the secession of the macroconidia from the parent hypha by autolysis of detaching cells, which is a similar manner of conidial detachment for many other dermatophytes [see, ref 2;3,4], and the separation of the detached macroconidia into compartments (Figs 4,5). Microconidia (and probably macroconidia) of *T mentagrophytes* are known to develop as a holothallic mode [see, ref. 2]. Our preliminary observations suggested the possibility of holothallic macroconidia changed by globular formation of macroconidial segments to several (arthric) conidia. However, the precise mode of their conversion remains to be clarified.

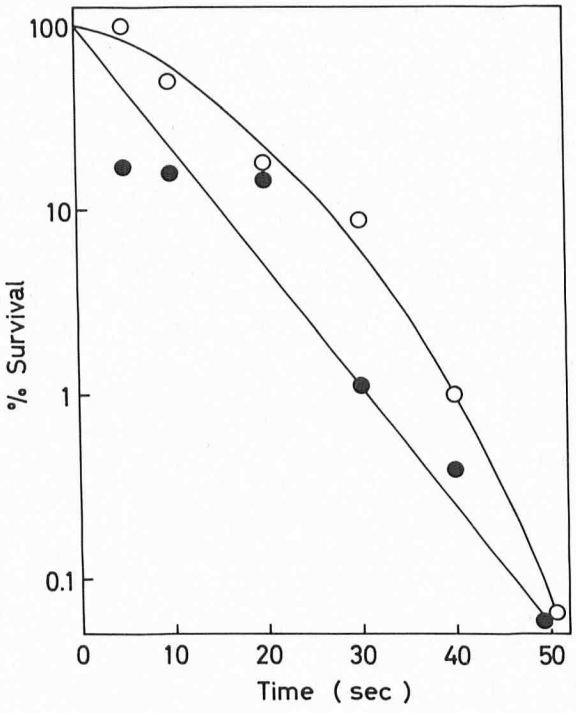

Figure 8. Ultraviolet sensitivities of microconidia (solid circles) and macroconidia (empty circles). *T mentagrophytes* microconidia were obtained from two-week cultures on Sabouraud glucose agar. Macroconidia were obtained from two-week cultures on Sabouraud glucose NaCl agar.

The present study has provided some new and significant information for the germination of macroconidia in *T mentagrophytes*. The following points must be noted concerning this matter. First, the retention of germination ability for long periods of time, apparently because of resistance of drying, suggests the potential of the macroconidia as infectious agents for humans and animals. Furthermore, the observation that a number of macroconidia germinated even in 10% NaCl implies their high salt tolerance. Incidentally, Hashimoto and Blumenthal [9] demonstrated that arthroconidia, which are produced during active *T mentagrophytes* infection, were quite susceptible to desiccation. Second, there is a difference in optimum temperature for the germination of macroconidia between *T mentagrophytes* and *M gypseum:* 37°C for the latter [6] versus 18–25°C for the former. In this connection, the optimum temperature for germination of *T mentagrophytes* arthroconidia is reported 37°C and various nutrients and pretreatments are required for germination [10]. The *T mentagrophytes* macroconidia, on the contrary, germinated even in distilled water or saline without any special activation (Table II). As is well known for many types of cells without lucid explanations, we confirmed the complete inhibition of macroconidial germination when densely populated conidial suspension was examined for germination still in a most appropriate medium (Table I). Third, judging from the freeze–thaw experiments, freezing at −70°C is suitable for macroconidial preservation. Although macroconidia and microconidia of *T mentagrophytes* differ in size and wall thickness, their susceptibilities to UV irradiation were almost the same. On the other hand, the arthroconidia of *T mentagrophytes* were reportedly very resistant to UV irradiation [9]. Overall, it may be speculated that macroconidia (and microconidia) are considerably different from arthroconidia in their responses to environmental conditions. As UV irradiation was effective for inactivation of macro- and microconidia, it could be used to sterilize contaminating areas such as locker room floors and mats. The differences in resistance to drying and optimum temperature for germination between arthroconidia and macroconidia might be a reflection of their distinct ecologic nature; the latter is produced as saprophytic conidia while the former as parasitic.

We are deeply indebted to Dr. H. Nakayama of Kyushu University for a critical reading of the manuscript and helpful suggestions. We would also like to thank H. Shimazu for helping photographic preparation.

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