

Scanning Electron Microscopy of *Malassezia Furfur* in Tinea Versicolor

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Morphologic features of Malassezia(M.) furfur in the horny layer from clinical lesions of tinea versicolor were examined by scanning electron microscopy and compared with the appearance of fungus in the horny layer from normal skin and in culture. In skin lesions of tinea versicolor, M. furfur showed a variety of growth and reproduction patterns. Although the main patterns were budding yeast forms, various patterns suggesting yeast-mycelial conversion were observed and mycelial hyphae were more prominent in the deeper horny layer than in the superficial layers. However, in the skin of normal persons and in culture, M. furfur existed only as yeast forms and no mycelial hyphae or yeast-mycelial conversion forms were seen. This suggests that the morphologic change of M. furfur, from a yeast form to a mycelial hypha one, may play a role in the induction of the clinical lesion of tinea versicolor.

Key Words: *Malassezia furfur*, tinea versicolor, scanning electron microscopy

Malassezia(M.) furfur is not only a saprophyte which presents in normal human skin but is also the etiological agent of tinea versicolor (Gordon 1951; Burke 1961; Roberts 1969; Faergemann and Bernander 1979). The factors which contribute to the transformation of the saprophyte into a pathogenic organism are still not defined.

M. furfur is a dimorphic fungus capable of yeast-mycelial conversion. The short hyphae and round yeast cells are characteristic in the parasitic stage (McGinley *et al.* 1970). The hyphae may be important in the parasitic stage which manifests itself clinically as the skin lesion of tinea versicolor (Tosti *et al.* 1972; Barnes *et al.* 1973).

This study was performed to investigate the morphologic features of *M. furfur* concerning yeast-mycelial change in lesions of tinea versicolor compared to those in normal skin and in cultures by scanning electron microscopy.

MATERIALS AND METHODS

Skin surface biopsies were performed in 6 healthy

adult males who had no internal problems except lesions of tinea versicolor on the trunk and extremities and also in 6 normal persons using cyanoacrylate adhesive by the method of Marks and Dawber (1971). For *in vitro* culture, the biopsy specimens were inoculated in a media which was composed of neopeptone 10g/l, Bactoagar 18g/l, glucose 40g/l, yeast extract 0.1g/l, glycerol monostearate 2.5g/l, Tween 80 20ml/l, olive oil 20ml/l, chloramphenicol 50mg/l, and cycloheximide 500mg/l and the pH was adjusted to 6.0. After 7 days of incubation at 37°C, cultures were prepared using vapor fixation and a simple vapor diffusion dehydration apparatus to minimize the disturbance of the delicate structure of fungus grown on agar media for SEM (King and Brown 1983). Unfixed preparations were air dried and coated with gold. The samples were examined with a Hitachi S-450 scanning electron microscope operating at 15 or 20 KeV.

RESULTS

Clusters of spheroidal cells and short hyphae were observed in the nest-like cavities. Apical buds which are produced by reproductive activity extruded from the surface of yeast cells (Fig. 1A). Growth and reproduction patterns of *M. furfur* are variable and the main characteristics are round, ovoid, or bottle-shaped yeast cells. Filamentation in hyphae is also a common feature of the fungus in clinical lesions (Fig. 2A). Some patterns suggesting yeast-mycelial conver-

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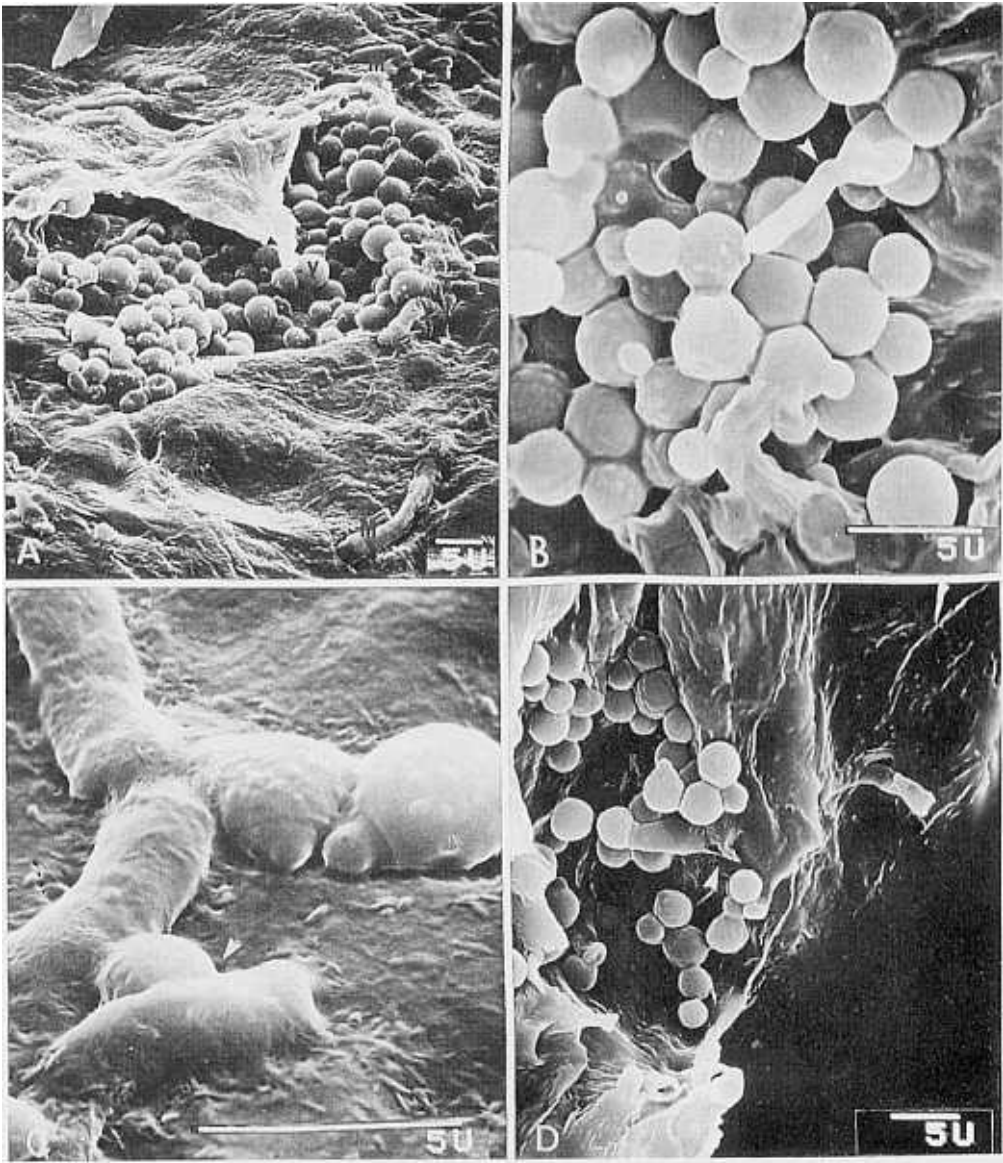


Fig. 1. *Malassezia furfur* in the superficial portion of clinical lesions of tinea versicolor. A, Clusters of yeast cells (Y) and mycelial hyphae (M) were seen in the nest-like cavity of the horny layer. B, Hyphae development of a yeast cell. C, Lateral budding from a hypha. D, Penetration of hyphae into the horny cells.

sion were present: hyphae development from a yeast cell (Fig. 1B), lateral budding from a hypha (Fig. 1C), and spore extrusion from hyphae (Fig. 2B).

In the horny layer, the hyphae which developed from yeast cells penetrated distally into the horny cells (Fig. 1D), and the mycelial hyphae meandered into the horny layer to reach levels deeper than those of the clustered yeast cells and to form new cavities

coated with thin horny film (Fig. 2A). From these hyphae, yeast cells were extruded and new clusters were formed by budding (Fig. 3). The clusters were larger and more numerous in the superficial horny layer than in the deeper layers where mycelial hyphae were interspersed with yeast cells. In the horny layer of normal persons, the clusters were composed of only yeast forms and were low in number compared

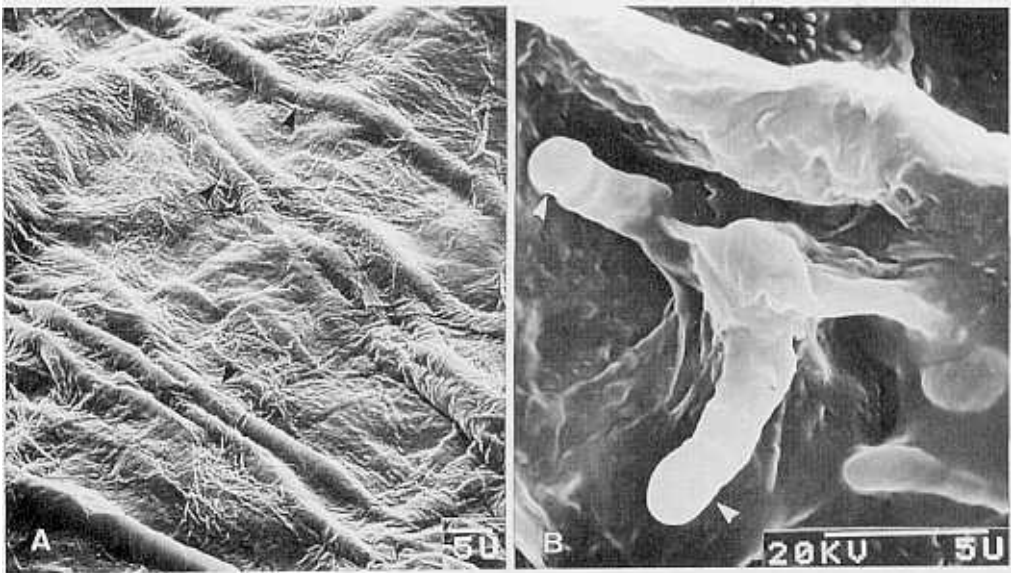


Fig. 2. *Malassezia furfur* in the middle portion of clinical lesions of tinea versicolor. A, Filamentation from a hypha coated by a thin horny film. B, Yeast extrusion from the apical portions of hyphae.

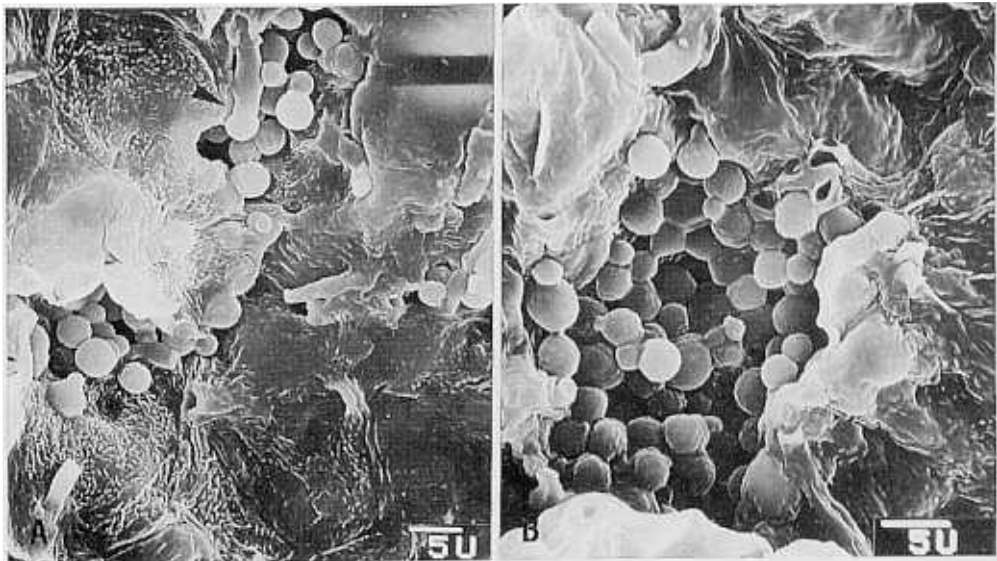


Fig. 3. Newly formed cluster by invading hyphae(A) shows numerous budding yeast cells(B).

to the cluster of the lesions of tinea versicolor (Fig. 4). They were observed mainly around hair follicles and adjacent areas. In culture, *M. furfur* also showed clusters of only yeast forms (Fig. 5).

DISCUSSION

M. furfur was authentically isolated and characterized in 1951 by Morris Gordon, who renamed it *Pityrosporum* (P.) orbiculare. It is now concluded that

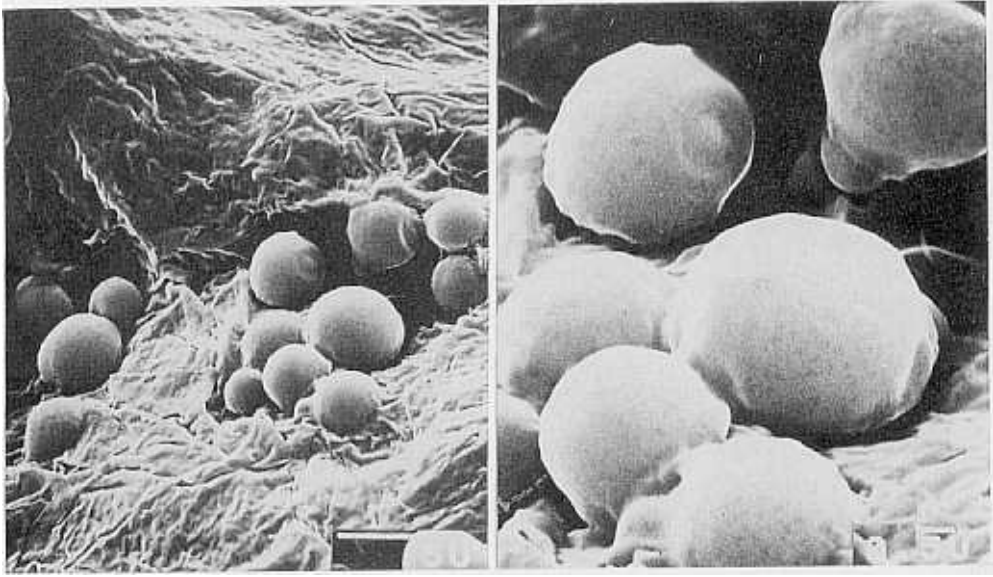


Fig. 4. In normal human skin, nests of only yeast cells without hyphae are seen.

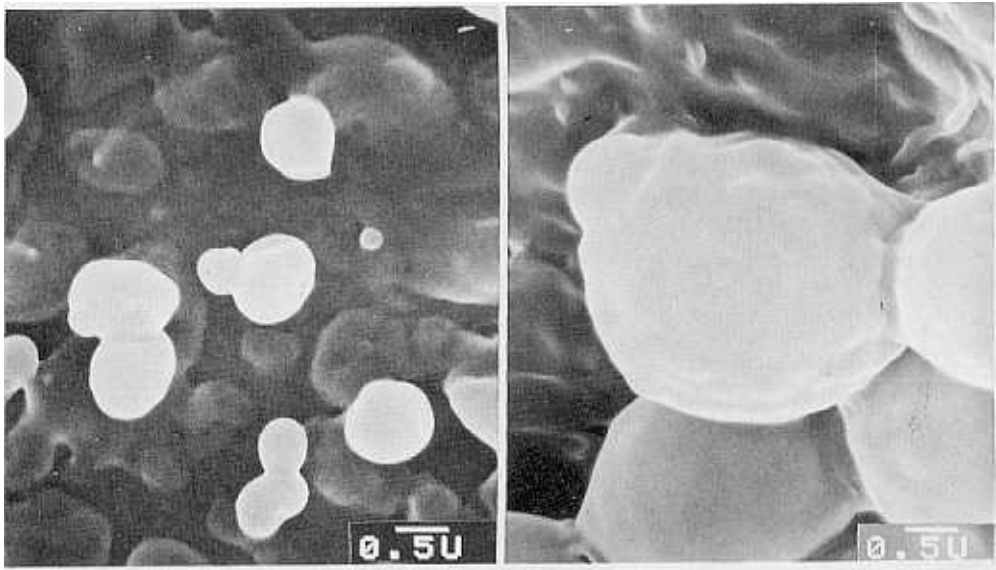


Fig. 5. *Malassezia furfur* from an artificial medium shows only budding yeast cells without hyphae.

M. furfur is the correct name for the agent of tinea versicolor and that *P. orbiculare* is a synonym of it (McGinley *et al.* 1970). *M. furfur* is not only the causative organism of tinea versicolor but also is normally found on the skin areas that are supplied with plenty of sebaceous glands (Noble and Sommerville 1974; Faergemann and Bernander 1981).

M. furfur is a dimorphic fungus showing yeast-mycelial conversion (Dorn and Roehnert 1977; Galimberti *et al.* 1987). In normal human skin, it is found usually on the trunk, flexural area, face and to a lesser extent on the scalp as cutaneous flora; hyphae are nearly always not seen and only clusters of yeast cells are found with incidental hyphae (Roberts 1969).

M. furfur shows only ovoid and bottle-shaped yeast cells with no tendency to develop hyphae in usual culture media and can show various shapes and sizes according to the culture condition (Sternberg and Keddie 1961; Keddie and Shadomy 1963). However, in skin lesions of tinea versicolor, the simultaneous presence of yeast and mycelial forms is usually observed. Therefore, the abundance of hyphae in the skin lesion of the disease is considered to have an important role in the parasitic stage (McGinley *et al.* 1970; Galimberti *et al.* 1987). We performed the present study to observe the behavioral patterns of *M. furfur* regarding yeast-mycelial transition in the saprophytic stage and the parasitic stage *in vivo* and in culture. The results in this study suggest that the mycelial hyphae form may be responsible for a clinical disease. Due to the growing nature of the horny cell layer in the epidermis, the clusters passively move to the outer surface and hyphae invade distally into the deeper portion. The lesions may persist between these well balanced kinetics.

The pathophysiologic states related to the transformation from yeast to the mycelial phase are not clarified completely. Several factors, such as hormonal factors including hypercortisolism, genetic or constitutional factors and climatic factors, are thought to be involved in this change (Burke 1961; Roberts 1969; Faergemann and Fredrikson 1981). Although the *in vitro* induction of the yeast-mycelial conversion has been unsuccessful so far in usual media, there have been reports of the *in vitro* production of hyphae of *M. furfur* in culture media with cholesterol and cholesterol esters (Porro *et al.* 1977). Their results suggest that abnormalities of cutaneous lipids may play an important role in the parasitic change. Further studies for the key factors contributing to the transformation of *M. furfur* will be needed.

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