Growth and Filament Production of *Pityrosporum orbiculare* and *P. ovale* on Human Stratum corneum *in vitro*

JAN FAERGEMANN, RAZA ALY and HOWARD I. MAIBACH

Department of Dermatology, University of California, School of Medicine, San Francisco, Ca 94143, USA

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When *Pityrosporum orbiculare* and *P. ovale* were incubated with stratum corneum epithelial cells, suspended in phosphate-buffered saline for 90 min at 37°C, short filaments were produced in 11–17.5 % of the yeast cells. A successful culture of *P. orbiculare* and *P. ovale* on human stratum corneum in vitro is described. When the stratum corneum pieces were incubated for 5 days in an environment with 7 % $C●_2$, a picture resembling that seen microscopically in tinea versicolor was observed. Filaments were produced in 39–48 % of *P. orbiculare* and *P. ovale* cells; the longest pseudohyphae, 40–60 µm, were produced by *P. ovale*. This culture method provides the possibility of studying the parasitic mycelium form of *P. orbiculare* and *P. ovale* in vitro. The influence of antimycotics, other drugs, and interaction with other microorganisms can be studied. *Key words: Pityrosporum orbiculare; P. ovale; Filament production; Stratum corneum; in vitro.* (Received December 23, 1983.)

H. I. Maibach, Department of Dermatology, University of California, School of Medicine, San Francisco, CA 94143, USA.

The lipophilic yeasts *Pityrosporum orbiculare* and *P. ovale* are probably different morphological forms of the same species (8, 9, 12, 16). Because they are listed in the literature as two separate species, we chose to continue with this nomenclature. They are members of the normal human cutaneous flora (5, 10, 13) and the etiological agent of tinea versicolor (8, 11, 12, 13). In earlier attempts to produce hyphae in *P. orbiculare* and *P. ovale* in vitro only a few yeast cells produced filaments (4, 5, 7, 12), and longer hyphae were seldom found (4, 7, 12). We have shown that incubation of *P. orbiculare* and *P. ovale* in environments with increased CO_2 concentration enhanced filament production (7).

Recently, in a study of the adherence of *Pityrosporum* to stratum corneum cells (in preparation), we observed that *P. orbiculare* and *P. ovale* produced filaments in a small number of yeast cells when incubated together with stratum corneum cells. Dermatophytes grow well on stratum corneum in vitro, and these in vitro models with dermatophytes have been used to study the production of antibiotics and interaction with bacteria (2, 15, 17).

This paper describes the growth and production of filaments in *P. orbiculare* and *P. ovale* on human stratum corneum in vitro. The effect of carbon dioxide on the production of hyphae is examined. The filament production of *P. orbiculare* and *P. ovale* when incubated together with stratum corneum epithelial cells was also studied.

MATERIALS AND METHODS

Fungi

P. orbiculare, ATCC numbers 42132, 44031 , and 44341, and *P. ovale*, ATCC No. 1452 and CBS No. 1878, were grown on a glucose–neopeptone–yeast extract agar medium, as described elsewhere (7), at 37°C.

Table I. *Number of short filaments produced by Pityrosporum orbiculare and P. ovale when they were incubated with stratum corneum epithelial cells for 90 min at 37°C*

Fungi	Number of filaments (%) Mean ± SE
P. orbiculare ATCC 42132	11.4±0.30
P. orbiculare ATCC 44031	14.9±0.91
P. orbiculare ATCC 44341	17.5±1.55
P. ovale ATCC 1452	12.7±0.81
P. ovale CBS 1878	16.0±1.99

Table II. *Number of filaments produced in Pityrosporum orbiculare and P. ovale when they were incubated, in environments with or without 7% CO_2, on human stratum corneum in vitro*

	Number of filaments (%) Mean ± SE
Incubation without CO_2	
P. orbiculare ATCC 44341	22.2±0.62
P. ovale CBS 1878	25.1±2.22
Incubation with 7% CO_2	
P. orbiculare ATCC 44341	38.6±2.27
P. ovale CBS 1878	48.1±3.11

Incubation with stratum corneum epithelial cells

Stratum corneum epithelial cells were collected from the skin of the forearm, by gently rubbing the skin, with a wooden applicator. The cells were suspended in phosphate-buffered saline (PBS), pH 7.2. The adherence method of Aly et al. (1) was used, with a few modifications. The stratum corneum epithelial cells were washed by membrane filters with a pore size of 10 μm (Millipore Corp., Beford, Mass, USA) and adjusted to 10^5 cells ml^{-1}. *P. orbiculare* and *P. ovale* cells, from a 4-day-old culture, were washed twice in PBS by centrifugation and adjusted to 10^7 cells ml^{-1}. One ml of stratum corneum cells and 1 ml of *P. orbiculare* and *P. ovale* cell suspensions were incubated in a shaking water bath for 90 min at 37°C. After incubation the cells were washed by membrane filtration as described above. Smears were made, stained with gram crystal violet for 30 sec, and examined under the light microscope at ×1000 magnification. In all experiments 25 epithelial cells were examined and the number of filament-producing yeast cells per total number of adherent yeasts was counted. Controls were fungal cells incubated in PBS alone.

Inoculation on human stratum corneum in vitro

Full-thickness cadaver skin was obtained early post mortem and kept deep frozen until used. Before use the subcutaneous fat was removed. The skin was heated to 60°C in water, for 3–4 min, whereupon the epidermis peeled easily with angled forceps. Epidermal specimens, approximately 1.5×3 cm, were incubated on a microscope slide in a Petri dish with 0.5% trypsin in PBS, for 3 h at 37°C. The activity of trypsin was blocked with human serum and the loosened epithelial cells washed away with PBS, leaving the isolated stratum corneum. This was mounted on a microscope slide in a Petri dish, into which a water-soaked filter paper had been previously inserted. The stratum corneum was ethylene oxide sterilized for 8 h with subsequent aeration of the gas for 12 h.

Suspensions of *P. orbiculare*, ATCC No. 44341, and *P. ovale*, CBS No. 1878, from a 4-day old culture, containing 10^8 cells ml^{-1} were prepared. Six drops, delivered by a microsyringe, were placed on the stratum corneum and spread over the entire surface. The filter paper dishes were moistened with sterile water and the Petri dishes sealed with paper adhesive. They were now incubated in parallel at 37°C in two incubators; one contained an atmosphere of 7% CO_2. The stratum corneum pieces were incubated separately for 2, 5, and 9 days and examined macro- and microscopically. In addition cultures were made from the stratum corneum on the medium earlier described (7). All experiments were performed in duplicate.

RESULTS

Incubation with stratum corneum epithelial cells

As described in another investigation (in preparation) *P. orbiculare* and *P. ovale* adhered to stratum corneum cells in a mean number of 6.8–9.4 per stratum corneum cell. Short filaments were noted in 11.4–17.5% of *P. orbicluare* and *P. ovale* yeast cells after incubation with stratum corneum cells for 90 min at 37°C (Table I). The filaments produced were short, only around 8–12 μm. Short filaments were seen in fewer than 0.5% of *P. orbiculare* (*P. ovale*) cells incubated in PBS alone.

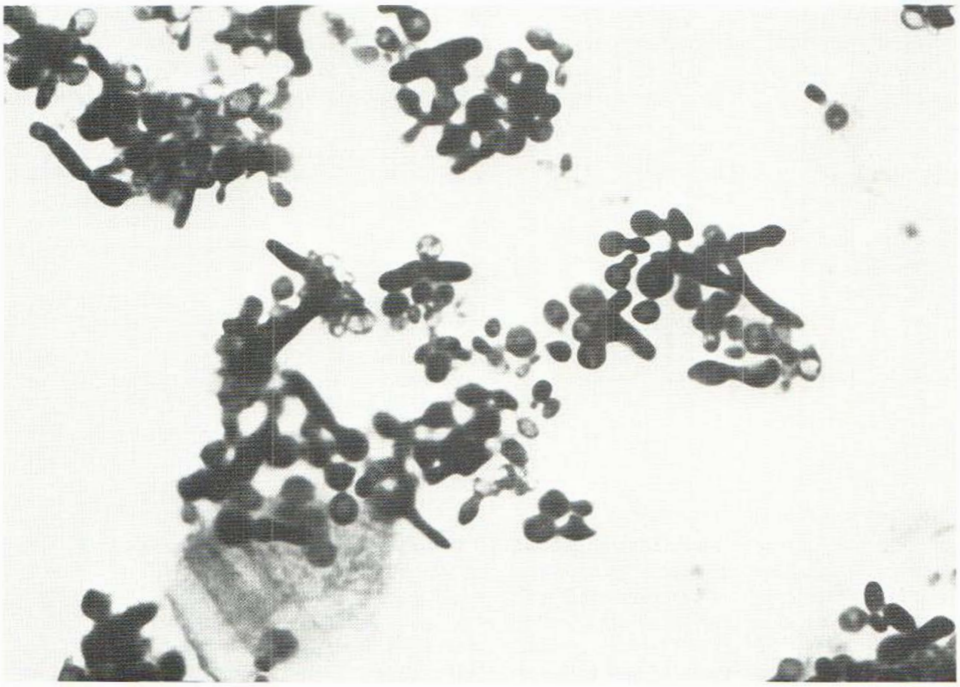

Fig. 1. Filaments produced by *Pityrosporum orbiculare* when grown on human stratum corneum, in an environment with 7% Co_2 in vitro (reduced from ×1 000).

Inoculation on human stratum corneum in vitro

P. orbiculare, ATCC No. 44341, and *P. ovale*, CBS No. 1878, were selected, because they produced the largest proportion of short filaments when they were incubated with stratum corneum epithelial cells. After 2 days' incubation, small colonies were observed on stratum corneum inoculated with *P. orbiculare* or *P. ovale*, and filaments were observed microscopically in several yeast cells; this was most pronounced when incubation took place in the added CO_2 environment. After 5 days, colonies of around 1–2 mm were seen. When incubation was in the CO_2 incubator, the number of yeast cells producing filaments had now increased to 39–48%. Microscopically, not only short filaments but also long pseudohyphae were produced in *P. orbiculare* (Fig. 1) and *P. ovale*. In *P. ovale* the hyphae were as long as 40–60 μm (Figs 2 and 3). In many areas, especially with *P. orbiculare*, the microscopic picture was indistinguishable from that seen in tinea versicolor. After 9 days, filaments were still present but now many yeast cells were swollen or showed other degenerative changes. Cultures taken from the stratum corneum were positive in all instances.

DISCUSSION

In tinea versicolor, *P. orbiculare* changes from its saprophytic yeast form to its pathogenic mycelium form (3,6). In primary cultures from tinea versicolor lesions, short filaments are often observed with *P. orbiculare* (3, 5, 14). When *P. orbiculare* and *P. ovale* are subcultured on routine media, no filaments are present (3, 5, 14). To study the pathogenic form of *P. orbiculare* (*P. ovale*) in vitro, a substrate which allows the organism to change to the filamentous form is desirable. In 1977 Dorn and Roehnert stated that they produced filaments in 7.5% of *P. orbiculare* cells when the organism was incubated on a defined medium containing glycine (4). Later Nazzaro Porro et al. reported filament production in approximately 20% of both *P. orbiculare* and *P. ovale* cells when the organisms were incubated on a medium containing cholesterol and cholesterol esters (12). We have earlier

Fig. 2. and 3. Filament production by *Pityrosporum ovale* grown in an environment with 7% CO_2 on human stratum corneum in vitro; observe the long pseudohyphae (reduced from ×1 000).

produced short filaments in *P. orbiculare* and *P. ovale* when incubated in environments with increased CO_2 concentration (5,7).

In the present investigation, short filaments were produced in both *P. orbiculare* and *P. ovale* in 11–17.5% of the cells, when they were incubated with stratum corneum epithelial

cells. Why the yeast cells starts to produce filaments after contact with stratum corneum cells remains unclear; receptor contact and/or substances released from the stratum corneum cells might be the cause of the filamentation.

This is the first report of growth of *P. orbiculare* and *P. ovale* on stratum corneum in vitro. The microscopic picture was similar to that seen in tinea versicolor. When incubation was in an environment with 7% CO_2, long pseudohyphae were seen; the longest produced by *P. ovale*. With this culture method it is now possible to study the pathogenic form of *P. orbiculare* and *P. ovale*. It offers the possibility of investigating the influence of antimycotics and other drugs on the mycelium form of *P. orbiculare* and *P. ovale*, and to compare the results with those obtained in the yeast form seen on other media. The interaction between the mycelium form of *P. orbiculare* (*P. ovale*) and other microorganisms can be studied.

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