

## Anthralin is a Potent Inhibitor of *Pityrosporum orbiculare/Ovale* *In vitro*

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**Two strains of *Pityrosporum orbiculare/ovale* were grown in a liquid medium and exposed to different concentrations of the imidazoles ketoconazole and clotrimazole as well as anthralin, liquor carbonis detergens and salicylic acid. With regard to growth inhibition of yeast cells, the efficacies of anthralin and the imidazoles were similar, a half-maximal inhibition being achieved with an anthralin concentration of 7 mg/l. Liquor carbonis detergens and salicylic acid also inhibited growth of *Pityrosporum orbiculare/ovale*, but only at much higher concentrations. The response to salicylic acid was mainly due to its acid pH. Key words: Imidazoles; Salicylic acid; Growth inhibition.**

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Yeasts of the genus *Pityrosporum* colonize healthy human skin as saprophytes, but are also the causative agents of pityriasis versicolor (1), *Pityrosporum* folliculitis (2) and *Malassezia intertrigo* (3). A pathogenetic role of *Pityrosporum orbiculare/ovale* in seborrheic dermatitis was elucidated especially by Faergemann and co-workers (4-6) and has also been proposed for psoriasis (7, 8), atopic eczema (4) and papillomatosis confluens et reticularis (9). One argument for this hypothesis was the response of the above-mentioned diseases to antimycotics (7, 10, 11). To our knowledge only little attention has been paid to the effect of classical antipsoriatic or antiseborrheic agents on *Pityrosporum*. Therefore, the aim of the present study was to investigate the influence of several primarily non-antimycotic drugs, which are frequently used for external therapy of those skin diseases, on the growth of *Pityrosporum* *in vitro* and compare it with that of true antimycotics.

### MATERIAL AND METHODS

Two strains of *Pityrosporum orbiculare/ovale*, CBS 6001 and CBS 1878, which originated from the Centraalbureau voor Schimmelcultures, Delft, Netherlands, were used for the study. The growth medium consisted of Sabouraud 2% glucose broth (Merck, Darmstadt, F.R.G.) containing 7.5% Tween 80 (Serva Feinbiochemica, Heidelberg, F.R.G.), 0.25% glycerol monostearate (Sigma Chemical Company, St. Louis, USA) and 0.1% yeast extract (Merck), pH 5.7. Ketoconazole, clotrimazole, anthralin, liquor carbonis detergens and salicylic acid were added to the growth medium at various concentrations. The latter two substances were dissolved directly in the medium. The imidazoles and anthralin were added to the medium after dissolution in acetone. The final concentration of acetone in the medium

did not exceed 0.5%. In these experiments, acetone was also added to the controls.

Since salicylic acid caused a drop in the pH-value of the medium to 3.4 at a concentration of 10 g/l, these experiments were repeated after adjustment of the pH-value to 5.7 by the addition of HEPES buffer (Sigma Chemical Company). The other agents did not influence the pH of the medium in the concentrations tested.

Two-ml aliquots containing increasing concentrations of the tested agents were incubated with 100,000 cells of the strains CBS 6001 or CBS 1878, respectively, at 32°C in the dark under constant shaking for 18 h. After the incubation period the cells were counted in a Neubauer chamber. All experiments were performed in triplicate at least. The two tested strains did not show any differences in their response. The concentrations giving half-maximal growth inhibition of *Pityrosporum* were evaluated from dose-response curves.

### RESULTS

Exclusively blastospores and no mycelial elements grew in the cultures. In contrast to most of the recommended media, which contain olive oil, the medium used was free from oil and lipid droplets, which facilitated the counting of the cells.

All tested agents inhibited the growth of *Pityrosporum* species. The imidazoles ketoconazole and clotrimazole showed half-maximal growth inhibition at concentrations of 0.8 and 45 mg/l, respectively (Table I). Among the other agents, anthralin turned out to be the most effective, with half-maximal growth inhibition at 7 mg/l and nearly complete inhibition at 20 mg/l. However, no fungicidal effect on *Pityrosporum* was exerted by anthralin up to the highest tested concentration of 100 mg/l. For salicylic acid and liquor carbonis detergens, much higher concentrations were required to achieve half-maximal growth inhibition, viz 400 and 1,850 mg/l, respectively.

The addition of salicylic acid reduced the pH of the medium at the lowest concentration (200 mg/l) from 5.7 to 5.2 and at the highest concentration (10 g/dl) to 3.4. In a second assay the pH was kept constant at 5.7 by adding HEPES buffer. In this case the inhibitory effect of salicylic acid on the growth of *Pityrosporum* was much weaker, achieving a half-maximal inhibition at approximately 10 g/l.

Table I. Concentrations causing half-maximal growth inhibition of *Pityrosporum orbiculare/ovale*

Ketoconazole	0.8	mg/l
Anthralin	7	mg/l
Clotrimazole	45	mg/l
Liquor carbonis detergens	1,850	mg/l
Salicylic acid (unbuffered)	400	mg/l
Salicylic acid (buffered at pH 5.7) approx.	10,000	mg/l

## DISCUSSION

In the present study we compared the effects of some classical antipsoriatic and antiseborrheic agents with those of well-known antimycotics on *Pityrosporum orbiculare/ovale*. We used the imidazole antimycotics ketoconazole and clotrimazole, which showed half-maximal growth inhibition of *Pityrosporum* at 0.8 and 45 mg/l, respectively. Our results concerning ketoconazole are in keeping with those of Faergemann & Borges (12), who used a growth medium completely different from ours. Compared with data from the literature, the efficacy of clotrimazole was lower in our experiments, possibly due to a considerable variation in sensitivity of different *Pityrosporum* strains to this particular antimycotic (13).

Anthralin exhibited a half-maximal inhibition at a concentration of 7 mg/l, which means that the efficacy of anthralin is comparable to that of the imidazoles tested. For comparison, the lowest anthralin concentration in ointments for topical treatment of psoriasis or seborrheic dermatitis is usually 500 mg/kg, which is 70-fold higher than the anthralin concentration causing half-maximal growth inhibition of *Pityrosporum* in vitro. Therefore we conclude that therapeutic concentrations of anthralin will strongly inhibit *Pityrosporum*, which is presumed to play a role in seborrheic dermatitis (4–6) and Koebnerization of psoriasis (7, 8, 14).

The two other antipsoriatic agents tested in this study, liquor carbonis detergens and salicylic acid, also inhibited the growth of *Pityrosporum*, though they were much less effective than anthralin. Half-maximal inhibition was achieved at a concentration of 1.85 g/l for liquor carbonis detergens and at 0.4 g/l for salicylic acid. Nevertheless, the usual therapeutic concentrations of these agents, i.e. approximately 5–20% for liquor carbonis detergens and 3–5% for salicylic acid, may also be able to inhibit the growth of *Pityrosporum* in vivo. In the case of salicylic acid, its inhibitory effect due to the lowering of the pH might be neutralized by the buffer capacity of the skin.

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