# In Vitro Adherence of *Candida Albicans* to Human Corneocytes

## *Inhibition by Chitin-Soluble Extract*

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In vitro adherence of *Candida albicans* to human corneocytes and the effect of a chitin-soluble extract (CSE) on the adherence reaction were studied. Adherence of the yeasts to cells obtained from different individuals was variable. However, repeated adherence tests with pooled corneocytes of 2 individuals from this group showed that the adherence parameters did not differ greatly throughout these tests. CSE at the concentration of 50 mg/ml had a significant inhibitory effect on the attachment of *C. albicans* to the corneocytes, most probably by blocking their receptors of attachment. The data indicate that the preparation may be useful in the prophylactive management of recurrent cutaneous candidiasis in susceptible individuals. (Received August 5, 1987.)

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Colonization and infection of the skin by Candida species is associated predominantly with *C. albicans* and only rarely with other Candida species (1). The organism is a rare inhabitant of the skin in healthy humans (2). Cutaneous candidiasis usually originates from endogenous organisms, which have saprophytically colonized epithelial surfaces, and opportunistically became pathogenic. Adherence to the epithelial surface may be the first step by which *C. albicans* can produce infection of the skin (1, 3).

Numerous studies have demonstrated that microorganisms adhere to epithelial surfaces via certain receptors. Adhesion can be blocked by the application of substances which mimick mammalian or microbial receptors (4–7). It has also been well demonstrated that *C. albicans* adheres to human vaginal (8–9), buccal mucosal cells and corneocytes (1, 3). The mechanisms of adherence of *C. albicans* to corneocytes and to the mucous membranes are probably similar (3). It was shown by Collins-Lech et al., that various aminosugars inhibit the in vitro adherence of *C. albicans* to human corneocytes (3). In previous studies we found that chitin and its derivatives may be involved in the binding of *C. albicans* to vaginal epithelial cells (8) and that a soluble product derived from chitin (Chitin-Soluble Extract, CSE), had an inhibitory effect, both in vitro and in vivo, on the attachment of *C. albicans* to vaginal mucosa (9) and in vitro to mouse intestinal mucosa (10). The present study was designed to investigate the in-vitro effect of CSE on the adherence of *C. albicans* to human corneocytes.

## MATERIALS AND METHODS

### *Yeast culture*

*C. albicans* CBS 562 was used throughout this study. The conditions for growth of yeasts have been described previously (8). Briefly, yeasts were grown overnight in liquid yeast extract medium (YE)

(Difco) at 28°C under constant shaking. The organisms were washed twice in phosphate-buffered saline (PBS) and resuspended in PBS to the desired concentration (up to $10^8$/ml).

*Corneocytes*

Human corneocytes were obtained from normal, healthy volunteers by gently scraping the flexor area of the forearm with a Teflon policeman. The scraped area was then gently rubbed with a cotton swab dipped in 0.1% Triton X-100 in PBS. The procedure was repeated, the cells were pooled, washed twice, counted and suspended to the desired concentration (up to $10^6$/ml).

*In vitro adherence reaction*

The in vitro adherence reaction was carried out by a modification of the technique described previously for vaginal cells (8). Briefly, equal volumes (0.2 ml of corneocytes ($10^6$/ml) suspended in 0.1% Triton X-100 in PBS and yeasts ($10^8$/ml) suspended in PBS were mixed and incubated on a rotator at 37°C for 2 h. Adherence was assayed microscopically. Corneocytes with five or more yeasts on their surface were considered to be adhesive cells. The percentage of adherence was determined by counting the number of corneocytes with adherent yeasts out of a total of 50 cells. In addition, the total number of adherent yeasts was also counted.

*Preparation of chitin-soluble extract (CSE)*

A 20% chitin (Sigma) suspension in sterile distilled water was incubated at room temperature for 5 h under constant shaking. The supernatant, designated CSE, was then removed, dialysed overnight at 4°C against sterile distilled water and lyophilized. Inhibition experiments were performed with the lyophilized material at various concentrations.

*Inhibition of in vitro adherence*

Inhibition of in vitro adherence was evaluated by assaying the percentage adherence in the presence of the potential inhibitor (CSE), as compared with a control (devoid of the inhibitor). Mixtures of corneocytes ($10^6$/ml) and yeasts ($10^8$/ml) were suspended in 0.4 ml of the inhibitor (at 25–50 mg/ml concentrations) and the adherence reaction was carried out as described before. The results of the experiments were analysed by the Student's *t*-test.

## RESULTS

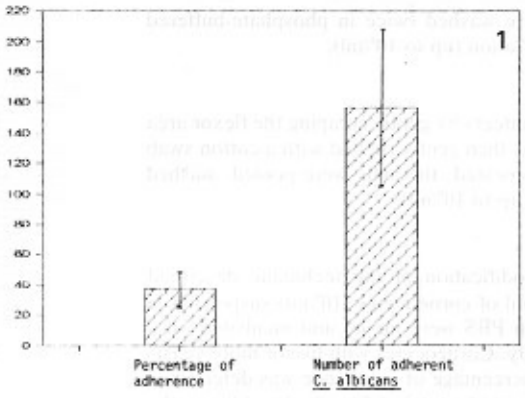
*Adherence of C. albicans to corneocytes from normal individuals*

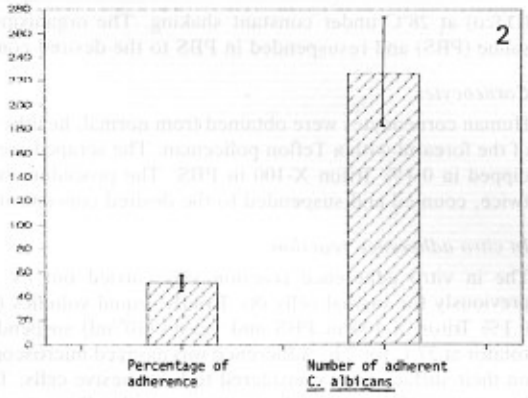
The attachment of *C. albicans* to corneocytes obtained from normal individuals was first determined in order to investigate the individual variability of the adherence reaction. Results of 24 adherence reactions, each in duplicate, with corneocytes from 22 healthy individuals are shown in Fig. 1. Percentage adherence was between 26.1 and 47.9 (mean 37). The number of adherent *C. albicans* was between 105.9 and 208.1 (mean 157).

Two individuals from the above-mentioned group who had relatively high values of both percentage adherence and number of adherent yeasts, were chosen for repeated adherence tests. The pooled corneocytes were tested independently six times, each in duplicate. The results of the repeated adherence tests with corneocytes of these 2 individuals are shown in Fig. 2. Percentage adherence was between 42.03 and 61.31 (mean 51.67). The number of adherent *C. albicans* was between 182.6 and 271.4 (mean 227). These results indicate that the variability of both parameters did not differ greatly during repeated adherence tests.

*Adherence of C. albicans to corneocytes in the presence of CSE*

The effect of CSE on adherence of *C. albicans* to corneocytes was tested. The concentration of 25 mg/ml of CSE was ineffective in causing inhibition of the adherence reaction, as opposed to 50 mg/ml which had an inhibitory effect. The results of 12 adherence reactions in the presence of 50 mg/ml CSE are delineated in Fig. 3. Percentage adherence was between 11.7 and 40.3 (mean 26), as compared with values between 44.6 and 73.4 (mean 59) in the control group. The number of the adherent yeasts was between 17.3 and 194.4



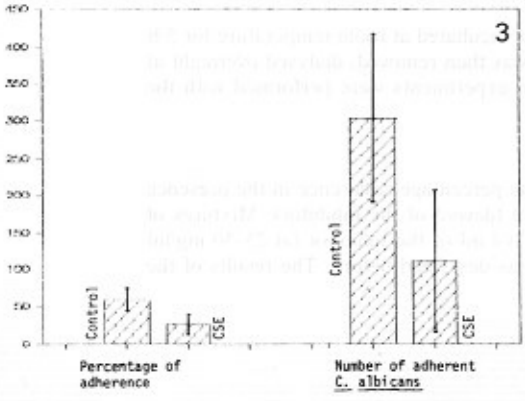




*Fig. 1.* Adherence of *C. albicans* to corneocytes from normal individuals. Mean values from 24 samples of corneocytes, collected from 22 individuals. Each sample was tested in duplicate. Number of adherent *C. albicans* denotes the total number of yeasts adhering to 50 corneocytes.

*Fig. 2.* Repeated adherence tests with corneocytes from the same donors. Means of values from six tests, each in duplicate, with a pool of corneocytes obtained from 2 individuals. Number of adherent *C. albicans* denote the total number of yeasts adhering to 50 corneocytes.

*Fig. 3.* Adherence of *C. albicans* to corneocytes in presence of CSE. Means of values from 12 experiments, each in duplicate. Analysis of data by Student's *t*-test reveals $p<0.001$ for both parameters. Number of adherent *C. albicans* denotes the total number of yeasts adhering to 50 corneocytes.

(mean 111.7) in comparison with the values of 190.8–415.8 (mean 303.3) in the control group.

*P*-values for both the percentage adherence and for the number of adherent *C. albicans* were less than 0.001, indicating that the difference between the test group (with CSE) and the control group are statistically significant.

## DISCUSSION

It has been reported that infection with certain microorganisms can be prevented by blocking the attachment of these microorganisms to host mucosal surfaces, by using analogues to host cell receptors or the adhesins of the microorganisms (4–6). In previous studies we demonstrated that chitin and its derivatives may be involved in the binding of *C. albicans* to epithelial cells (8), and that the soluble product derived from chitin (CSE) had an inhibitory effect on the in vitro attachment of the yeasts to human vaginal epithelial cells (9) and to mouse intestinal mucosal cells (10).

In the present study it was shown that CSE has an inhibitory effect on the in vitro attachment of *C. albicans* to human corneocytes.

Chitin, the polymer of the amino sugar *N*-acetyl-glucosamine, is present in the cell walls of blastospores and hyphae of *C. albicans* (11). A higher percentage of chitin was found in the mycelial form (12). Braun & Calderone (13) showed that the enzyme chitin synthetase is located in the plasma membrane of *C. albicans* as in other yeasts. It is also known that the

other polysaccharidic components of the Candida cell wall, maman and glucan, are linked to protein via *N*-acetyl-glucosamine (14). It has been postulated that the mechanisms of adherence of *C. albicans* to cells of the skin and mucous membranes are probably similar (1, 3). Our previous studies suggest that the effect of CSE on the adherence of *C. albicans* is most probably exerted by binding to the surface of the epithelial cells and thereby blocking their receptors of attachment. We presume that a similar mechanism may be involved in the inhibition of the adherence of *C. albicans* to corneocytes by CSE.

Cutaneous candidiasis usually affects the intertriginous areas of the skin. In those areas the warmth, moisture and maceration of the skin permit the organism to thrive. *C. albicans* is an opportunistic organism, able to behave as a pathogen generally only in the presence of an impaired host-immune response, rendering such individuals prone to the development of recurrent infections. Since adherence of the yeasts to the skin is believed to play a critical role in cutaneous candidiasis (1, 3), the preventive application of CSE over susceptible areas of the skin in patients with recurrent cutaneous candidiasis may be of great clinical value.

At present, the nature of the active component(s) in the CSE is still unknown. Thus, the exact characterization and the active concentration of the binding molecule cannot be accurately determined, which may explain the high standard deviation values, resulting from variations in the activity of the individual CSE preparations. Nevertheless the *p*-values for both the percentage of adherence and for the numbers of adherent yeasts ($p<0.001$) reveal the high significance of the data. Further experiments to characterize the active component(s) of CSE are currently in progress in our laboratory.

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