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Undecylenic Acid Inhibits Morphogenesis of *Candida albicans*

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Resilient liners are frequently used to treat denture stomatitis, a condition often associated with *Candida albicans* infections. Of 10 liners tested, 2 were found to inhibit the switch from the yeast form to hyphae and a third was found to stimulate this switch. The inhibitor was determined to be undecylenic acid.

Candida albicans is a significant opportunistic pathogen of humans and is a major cause of denture stomatitis, an inflammation of tissues underlying dentures. Adherence is critical to the pathology of *C. albicans*, serving as a first step of infection for many microorganisms (1, 2). In healthy mouths, saliva flow and scraping by the tongue limit the accumulation of microorganisms. Prostheses impair this flushing, facilitating establishment of a focal infection by retention of *C. albicans* close to the basal seat. *C. albicans* exhibits two cellular morphologies: the round yeast form, which is associated with asymptomatic carriage, and elongated hyphae, which are associated with active infections (9). Mutants incapable of forming hyphae exhibit reduced virulence (4, 6, 7, 14). Change in form can be induced by temperature, pH, or serum (9). We have examined the effect of denture liners on morphology.

Strains used in these experiments were American Type Culture Collection (ATCC) strains 18804 and 28367 and strain SC5314. Cultures were grown on YPD medium at 30 or 39°C (10). Cell densities were determined by microscopic enumeration or by the optical density at 600 nm (OD_{600}) of the cultures. The liners, listed in Table 1, were prepared as recommended by the manufacturer. Most liners were formed by mixing two components that were allowed to harden at room temperature for 10 min before transfer to culture tubes containing 2 ml of YPD. Molloplast B was prepared by staff of the Medical University of South Carolina College of Dental Medicine. PlastiLiner, a ready-to-use product, was cut to size immediately before use.

Cells were grown overnight at 30°C on YPD agar and harvested by scraping the cells from the plate and suspending in YPD broth. Two milliliters of cells at 10^6 cells/ml was distributed into tubes containing liner, approximately 0.5 g of liner per tube. The cultures were incubated at 39°C with agitation for 2 h; planktonic cells were analyzed microscopically to determine the percentage of cells with germ tubes. A germ tube is the first hyphal cell emerging from a yeast-form cell. In a few cases, microscopic examination of cells adhered to liners was performed following staining with Gram's safrinin, and the morphologies were found to be qualitatively similar to the corresponding planktonic cells. Germination was induced at 39°C rather than 37°C because, in our experience, the higher temperature resulted in more consistent induction of germ tubes.

Cells cultured at 39°C in the absence of liner quantitatively converted to germ tubes within 2 h. The presence of liners had differential effects on germ tube formation. Most products caused a reduction in the percentage of germ tubes. Due to limited effect and variability, neither the statistical significance of these results nor the effect of these products was determined. In contrast to the majority of the liners tested, Coe-Soft (CS), Coe-Comfort (CC), and Tokuyama Soft Reliner (TSR) had significant impact on morphology. The Coe products completely inhibited, while TSR stimulated, the formation of germ tubes (Table 2).

Hyphae do not arise by a change in the shape of a yeast-form cell. Instead, a yeast-form cell reproduces to form a hypha. One mechanism by which a liner can inhibit morphogenesis is by inhibition of proliferation. Growth rates were determined

TABLE 1. Liners used in study

Product	Manufacturer or supplier	Denture liner	Tissue conditioner	Availability	
				Prescription	Over the counter
Coe-Soft	GC America (Chicago, Ill.)	+		+	
Molloplast B	DETAX GmbH (Ettlingen, Germany)	+		+	
Perma Soft	Myerson (Chicago, Ill.)	+		+	
Tokuyama Soft Reliner	Tokuyama (Tokyo, Japan)	+		+	
Coe-Comfort	GC America (Chicago, Ill.)		+	+	
Lynal	Dentsply (Milford, Del.)		+	+	
Acryline 2	Menley & James (Horsham, Pa.)	+			+
Denturite	Brimm Laboratory (Tonawanda, N.Y.)	+			+
PlastiLiner	Brimm Laboratory (Tonawanda, N.Y.)	+			+

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TABLE 2. Effect of TSR on morphology

Strain	Experiment no.	Control[a] (no. of samples)	TSR[a] (no. of samples)	*P* value
28367	1	81.1 ± 3.19 (2)	96.5 ± 3.68 (2)	0.017
	2	72.9 ± 4.19 (2)	92.7 ± 4.12 (2)	0.054
	3[b]	22.2 ± 8.43 (2)	90.0 ± 4.40 (2)	0.005
	4[b]	26.9 ± 16.42 (2)	68.99 ± 3.63 (6)	0.006
18804	1[b]	14.35 ± 9.72 (2)	95.37 ± 0.93 (6)	0.000003
SC5314	1	80.87 ± 9.92 (2)	95.79 ± 1.3 (6)	0.023

[a] Percentage of cells exhibiting germ tubes ± standard deviation.
[b] Experiments in which control cultures exhibited poor hyphal induction but TSR cultures exhibited good to excellent induction.

for cells growing at 30°C in the presence and absence of various liners. The growth rate was the time required for the OD_{600} to double while the culture was growing exponentially. None of the liners had a significant effect on growth, indicating that the decrease in germ tubes was not due to growth inhibition.

Liners are hydrophobic, and hyphae are more hydrophobic than yeast cells. The absence of planktonic hyphae in the presence of liners could be due to preferential binding of hyphae to liners. Strain 28367 was grown in the presence and absence of CS at 39°C to induce hyphae. Planktonic cells in the control culture readily formed hyphae while all of the planktonic cells growing in the presence of CS were yeast-form cells. Microscopic examination of adherent cells revealed that all were yeast form. Thus, CS was affecting the ability of cells to change morphology rather than preferentially binding hyphae.

The basic composition of the Coe products is not significantly different from other acrylic liners. One distinct difference, however, is the inclusion of undecylenic acid (UDA) in the Coe products. UDA is present in CS and CC at approximately 70 mM (from the material safety data sheet supplied with the products). The ability of UDA to inhibit conversion of yeast cells to hyphae was tested by culturing cells in the presence of UDA under inducing conditions (39°C for 2 h). All three strains yielded similar results, but only data for strain 28367 are presented in Fig. 1 for simplicity. The presence of UDA inhibited the appearance of germ tubes (Fig. 1A), with 10 μM UDA causing a sevenfold reduction. This concentration of UDA had no effect on the growth rate, indicating that morphogenesis, not growth, was being inhibited (Fig. 1B). The concentration used here was much lower than that present in CC and CS. The high concentration in the liners was necessitated by the low bioavailability of the UDA within the liner matrix. These results confirm that UDA is likely to be the component of the Coe products responsible for inhibition of germ tube formation.

Cultivation of *C. albicans* at 39°C in the presence of TSR stimulated germ tube formation with all three strains tested in numerous independent experiments. In some experiments, control cultures exhibited poor induction of hyphae while TSR cultures exhibited nearly uniform induction. The increase in planktonic hyphae was not due to preferential adherence of yeast-form cells to TSR: microscopic examination of adherent cells revealed exclusively hyphae. The addition of 70 mM UDA to TSR was sufficient to inhibit the appearance of hyphae in cultures grown at 39°C, further implicating UDA in the inhibition of germ tube formation.

The effect of liners on morphology is significant because of the correlation between virulence and hyphae (4, 6, 7, 14). UDA is not the first antifungal agent shown to inhibit morphogenesis at sublethal concentrations. Nystatin (3), amphotericin B (3), and triazoles (13) have been shown to inhibit germ tube formation. One possible mechanism for UDA is the inhibition of enzymes involved in lipid metabolism. Cerulenin, an inhibitor of fatty acid biosynthesis, inhibited germ tube formation (5), and phospholipase D1 has been implicated in morphogenesis (8). Medium-chain fatty acids have been shown to disrupt the regulation of cytoplasmic pH by carrying protons across the plasma membrane (11). Such disruption by UDA could interfere with the alkalinization of the cytoplasm which accompanies germ tube formation (12).

Three points regarding TSR have clinical implications. First, liners are used to treat infected tissue. Second, hyphae are associated with an active infection. Finally, mutants that are unable to form hyphae exhibit reduced virulence (4, 6, 7, 14). Although the ability to form hyphae is not the only virulence factor of *C. albicans*, a denture liner that stimulates the production of hyphal cells could exacerbate the condition that it is intended to treat.

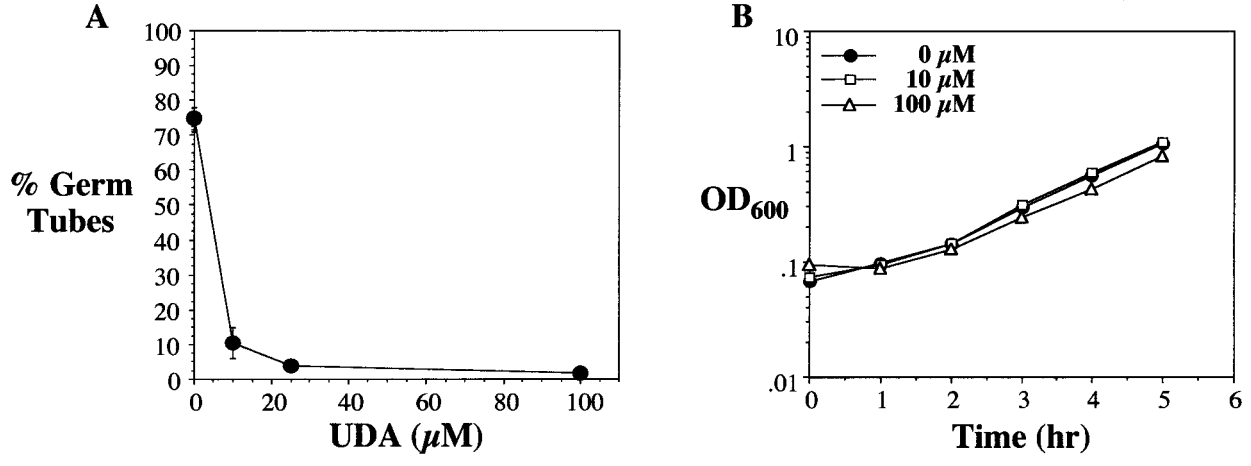

FIG. 1. UDA inhibits the formation of germ tubes. (A) Yeast cells were incubated in rich medium supplemented with the indicated concentrations of UDA at 39°C for 3 h. Cells were examined microscopically to determine the percentage of cells that had formed germ tubes. Error bars indicate the standard error of mean (n = 8 for each condition). (B) Growth curves for parallel cultures grown at 30°C in the presence of the indicated concentrations of UDA. The results of a single representative experiment are shown.

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