

***In vitro* studies on *Candida*, antimycotics  
and oral defences**

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## Preface

Research work for this thesis was carried out in the division of Oral Bio-Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong. The work is a *bona fide* product of the original research undertaken individually by the author. In the completion of the work, the author has directly supervised any assistance from technical staff from time to time.

Most of the findings presented in this thesis have been published in scientific journals, submitted for publication, or have been reported at scientific meetings. Research output from this study thus carried elsewhere is listed below:

### Publications

1. **Anil S**, Ellepola AN, Samaranayake LP. Post-antifungal effect of polyene, azole and DNA-analogue agents against oral *Candida albicans* and *Candida tropicalis* isolates in HIV disease. *J Oral Pathol Med* 2001; **30**(8): 481-8.
2. **Anil S**, Ellepola AN, Samaranayake LP. The impact of polyene, azole, and DNA analogue antimycotics on the cell surface hydrophobicity of *Candida albicans* and *Candida tropicalis* in HIV infection. *Mycopathologia* 2002; **153**(4): 179-85.
3. **Anil S**, Samaranayake LP. Impact of lysozyme and lactoferrin on oral *Candida* isolates exposed to polyene antimycotics and fluconazole. *Oral Dis* 2002; **8** (4): 199-206.
4. **Anil S**, Samaranayake LP. Brief exposure to antimycotics reduces the extracellular phospholipase activity of *Candida albicans* and *Candida tropicalis*. *Chemotherapy* (accepted for publication).

### Presentations at scientific meeting

**Anil S**, Ellepola AN, Samaranayake LP. Impact of lysozyme and lactoferrin on *Candida* species pre-exposed to antimycotics. *J Dent Res* 2001; **80**: (Special issue IADR Abstracts): 725. (Presented at the 79<sup>th</sup> IADR meeting at Chiba, Japan, June 27-30, 2001).

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Abstract of thesis entitled  
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**Abstract**

There is scant information on the effect of exposure to low dilutions of antifungal agents on the virulence attributes of the human fungal pathogen *Candida*, such as its cell surface hydrophobicity, and phospholipase and haemolysin production. Further, the impact of host mucosal antifungal proteins such as lactoferrin and lysozyme on drug-exposed *Candida* species has been little studied.

In order to obtain basic data on the latter aspects of *Candida*, *in vitro* evaluation of the post-antifungal effect (PAFE) of two polyenes (nystatin and amphotericin B), two-azoles (fluconazole and ketoconazole) and a single DNA-analogue (5-fluorocytosine) against ten oral isolates each of *Candida albicans* and *Candida tropicalis*, all from human immunodeficiency virus infected individuals, was performed. The term PAFE is given to the suppression of fungal growth that persists after limited exposure to a low dilution of an antifungal agent. One-hour exposure to twice the minimum inhibitory concentration of all the drugs, except fluconazole, elicited a consistently high PAFE in both *Candida* species. Furthermore, the PAFE elicited by the antifungals (except fluconazole) was significantly prolonged for *C. tropicalis* compared with *C. albicans*. The speedy recovery of *C. albicans* isolates exposed to transient, low concentrations of antifungals appears to be a reflection of its higher virulence. This data, while confirming the existence of PAFE in a non-*albicans* species of *Candida*, provided further clues for the recalcitrance of *C. albicans* infections in the face of antifungal therapy.

The cell surface hydrophobicity (CSH) of *Candida* is considered a critical factor contributing to its colonization potential and virulence. Comparison of the CSH of

the aforementioned *Candida* isolates following their brief exposure to nystatin, amphotericin B, ketoconazole, fluconazole and 5-fluorocytosine showed that the CSH of *C. albicans* is reduced to a significant extent when exposed to the antifungals, illustrating another mode of action of antifungals on *Candida* species that modify their colonization potential.

On investigating the *in vitro* phospholipase activity of *Candida* exposed to the foregoing antifungals, it was evident that *C. albicans* continued to produce higher levels of phospholipase than *C. tropicalis*. Furthermore, pre-exposure of all the isolates to antimycotics led to a significant reduction in the phospholipase activity. Similarly, all studied *Candida* isolates exposed to nystatin, amphotericin B and fluconazole showed a significant reduction in haemolysin production. This reduced haemolytic activity, evaluated using a novel plate assay, was maximum for nystatin-exposed yeasts followed by amphotericin B- and fluconazole-exposed *Candida*.

The antifungal effect of mucosal defense proteins lysozyme and lactoferrin on drug-exposed *Candida* species is not known. Following brief exposure to sub-therapeutic concentrations of the polyenes and fluconazole a significant increased yeast-susceptibility to lysozyme, but not to lactoferrin, was noted. Polyenes had a lesser impact on the lysozyme susceptibility of yeasts, compared with the azoles. Taken together, these data shed light on the mechanisms by which low dilutions of antifungal agents suppress the virulence attributes of *Candida* species whilst reinforcing the host mucosal defences. Further studies are warranted to examine whether these *in vitro* effects operate *in vivo*.