Characterisation of *Candida parapsilosis* infection of an *in vitro* reconstituted human oral epithelium

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Objectives: A variety of *Candida* spp can colonise the oral mucosal surface and co-exist as harmless commensals. However, if the host becomes debilitated, as seen in individuals with HIV infection, diabetes mellitus or those receiving drug therapy and broad spectrum antibiotics, candidosis can occur. *Candida albicans* is the most frequently isolated species from oral candidosis, although it is becoming increasingly evident that other Non-*C. albicans Candida* (NCAC) spp such as *Candida parapsilosis* are emerging as important human pathogens. Despite the recognition of *C. parapsilosis* as an opportunistic pathogen the genetic and virulence factors that enable *C. parapsilosis* to cause disease remain poorly understood. Increased awareness of such factors is important to facilitate the development of more effective management strategies against this organism. Hence, the objectives of this study were to assess the invasive capability of *C. parapsilosis* in a Reconstituted Human Oral Epithelium (RHOE) and to detect the expression of *SAP* genes during the invasion process.

Methods: *C. parapsilosis* originally recovered from the oral cavity (n=2), vagina (n=2), and urinary tract (n=2) together with *C. parapsilosis* ATCC 22019 were used to infect RHOE, which was incubated for 12 and 24h. One half of the tissue was fixed in paraformaldehyde and sectioned. The rehydrated sections (20µm) were then stained with concanavalin A–Alex 594 and Hoechst nucleic acid dye to assess *C. parapsilosis* colonization and invasion pattern using Confocal Scanning Laser Microscopy (CSLM). The remaining half of unfixed tissue was used for RNA extraction. This RNA together with broth culture extracts were subjected to RT-PCR targeting three secreted aspartly proteinases genes (*SAPP1-3*).

Results: CLSM revealed that all strains colonised the RHOE, although the extent was highly strain dependent as it was the morphology of the *C. parapsilosis* strains. After 12 h of incubation, *C. parapsilosis* invaded the upper three cell layers of the epithelium and it was evident some extensive epithelial damage after 24 h infection. Expression of *SAP* genes was also strain dependent with differences found between infecting and planktonically cultured *Candida*. Reduced expression of *SAPP3,* and increased expression of *SAPP1* and *SAPP2* for infecting strains was detected.

Conclusions: This work confirmed the effectiveness of RHOE as an *in vitro* model to study *Candida* virulence attributes and conclusively demonstrated that *C. parapsilosis*, whilst not highly invasive was able to induce significant damage to the tissue structure. *SAPP1* and *SAPP2* could be contributing factors in the invasion process and causing RHOE damage.