# Differences in activity profiles between biofilm and planktonic cells of Non-*Candida albicans Candida* species

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Infections caused by *Candida* species have increased greatly in the past decade and this has been associated with an increase in the elderly population base, an increase in the number of AIDS and immunocompromised patients, and an increasing use of indwelling medical devices. Most cases of candidosis are caused by *Candida albicans*, however, non-*Candida albicans Candida* (NCAC) species have also recently been identified as frequent pathogens. Examples of such NCAC species include *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida dubliniensis*.

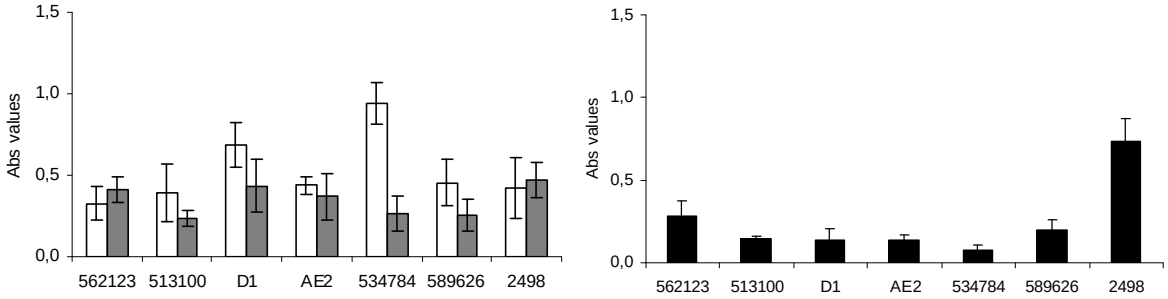
Biofilms are prevalent for environmental microbes and also play an important role in clinical infection. Biofilms have clinical significance as they exhibit elevated resistant to host defences and antimicrobial agents, therefore representing a persistent source of infectious organisms. Biofilms comprise cells embedded in a polymeric extracellular matrix and the methods used to quantify these cells tend to be based on indirect methods aimed at quantifying biomass (*e.g.* crystal violet staining) or cellular activity (tetrazolium salts reduction). It is generally accepted that cells behave differently in biofilms compared with their planktonic equivalents. Several researchers have demonstrated that *Candida* biofilms exhibit significantly enhanced resistance to commonly used antifungal agents, possibly due to alteration in activity. Thus, the aim of this work was to determine the relative activity of NCAC species in biofilm and planktonic states.

A total of 16 NCAC strains including *C. parapsilosis* (n=6), *C. glabrata* (n=6) and *C. tropicalis* (n=4), isolated from the vagina, urinary and oral tract were used. Reference strains of these species (*C. glabrata* IGG 2418, *C. tropicalis* IGC 3097T/CBS94, *C. parapsilosis* 37) were similarly tested. *Candida* were cultured on Sabouraud Dextrose Agar (SDA) for 24 h at 37ºC and then sub-cultured in Sabouraud Dextrose Broth (SDB; 37ºC, 130 rev/min, 18 h). These planktonic cells were harvested by centrifugation (5000 rev/min, 10 min at 4ºC) and washed twice with ultra-pure water. Quantification of planktonic activity involved suspending the cells ($1x10^8$ cells $ml^{-1}$) in tetrazolium salt solution (100 µg/µl XTT and 10 µg/µl phenazine methosulfate; PMS). The suspensions were incubated in

the dark for 3 h with agitation (130 rev/min) after which the absorbance was read at $490_{nm}$.

Biofilms were produced in 96-well microtire plates using an inoculum of $1x10^7$ cells/ml in SBD. Biofilms were prepared by shaking incubation (130 rev/min, 37ºC, 48 h) and quantified by total biomass measurement using crystal violet staining. As for planktonic cells, reduction of XTT was used to quantify activity. All tests were performed in triplicate.

Results showed that all NCAC strains produced biofilms, although there were differences depending on species, strain or isolate origin. *Candida glabrata* strains were generally less able to form biofilms compared with *C. parapsilosis* and. *C. tropicalis*. The results also suggested that there were intrinsic differences in cellular activity between strains, either in planktonic or biofilm forms. Figure 1 illustrates the biofilm forming ability and activity of *C. glabrata* strains. It was apparent that strain *C. glabrata* 534784 generated higher biofilm biomass whilst also displaying the lowest activity. Similarly, *C. glabrata* 534784 planktonic growth also had the lowest activity. Furthermore, it can be seen that planktonic cells of the *C. glabrata* reference strain (2498) had an activity significantly higher than clinical isolates. This difference was not evident for the biofilm activity of the reference strain.




(a) (b)

**Figure 1.** (a) Biofilms of *C. glabrata* strains formed in SDB after 48 h. (□) Absorbance values (570 nm) of crystal violet solutions and (■) absorbance values (490 nm) of XTT solutions. (b) Planktonic activity of *C. glabrata* strains. (■) Absorbance values (490 nm) of XTT solutions.

This study highlights the differences between *Candida* isolates in terms of their ability to form biofilms and their cellular activity. It can be inferred from the results that strain differences in both activity and biofilm formation occur. However, correlation between yeast activity in biofilms and planktonic modes of growth cannot readily be made.