

## Reference

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**Insights in non-*Candida albicans* *Candida* biofilms**

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**Abstract**

The number of infections caused by *Candida* spp has greatly increased in the past years, which has been attributed to an increase in the number of AIDS and immunocompromised patients, in the elderly population and the more frequent use of indwelling medical devices. Most Candidiasis have been attributed to *Candida albicans*, however, recently, non-*Candida albicans* *Candida* (NCAC) spp, as *C. parapsilosis*, *C. glabrata* and *C. tropicalis*, have been identified as common pathogens. Furthermore, *Candida* biofilm formation has important clinical repercussions due to their inherent tolerance to antifungal therapy and ability to withstand host immune defenses. Consequently, it is of utmost importance to understand the physiology and virulence of NCAC spp biofilms. Thus, the main aim of this work is to present some insights in *C. parapsilosis*, *C. glabrata* and *C. tropicalis* biofilms, through (i) biofilm characterization (structure and matrix composition); (ii) evaluation of antifungal agents tolerance and (iii) determination of putative virulence factors (extracellular enzymes and extracellular alcohols).

SEM observation of *Candida* spp biofilms revealed that biofilm architecture was neither species nor strain dependent. However, *C. glabrata* biofilms, which presented lower biomass, formed, generally, a more compact and thick structure than *C. tropicalis* and *C. parapsilosis* ones. Regarding matrix composition, *C. glabrata* presented, in general, higher amounts of proteins and polysaccharides, in opposition to *C. tropicalis* that presented lower amounts of both components. Biofilms antifungal resistance tests revealed that *C. glabrata* biofilms present high resistance to fluconazole and itraconazole, in comparison with the other NCAC spp biofilms.

With respect to putative virulence factors, the production of extracellular enzymes, namely proteases and phospholipases was also evaluated in *C. tropicalis* but there were no differences in the levels of enzymes production by biofilm and planktonic cells. Regarding the extracellular alcohols, it was found that *C. parapsilosis* and *C. tropicalis* produce farnesol, 1-dodecanol, 2-phenylethanol and isoamyl alcohol (already described for *C. albicans*). Furthermore, the latter was produced by *C. tropicalis* in a higher amount than by *C. parapsilosis*.

Overall, with this extensive research work it was possible to describe and relate several virulence features of NCAC spp with their putative virulence and invasiveness of human epithelia.