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**TITLE:** Game and player: *C. albicans* biofilm lifestyle and extracellular DNA

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**ABSTRACT**

DNA is as a structural component of bacterial biofilms extracellular matrix (ECM). Although evidences have shown that DNA may play a role in *C. albicans* biofilms, further studies are required to understand the contribution of extracellular DNA (eDNA) in *C. albicans* biofilm lifestyle. Herein we aimed to determine the eDNA content of *C. albicans* SC5314 biofilm ECM and the effect of DNase I and exogenous DNA treatments on biofilm formation and biofilm cells susceptibility to antifungals.
First, for eDNA estimation in *C. albicans* biofilm ECM, biofilms were formed under flow conditions for 48 h. ECM was isolated and its DNA and protein contents were determined. Second, DNase (0.02 - 2 mg/ml) and exogenous DNA (10 - 2560 ng/ml)

were added at different stages of biofilm development (microtiter plate model under static conditions). The effect of 24 h treatments was evaluated in terms of biofilm biomass by crystal violet assay ($A_{550}$). Third, for antifungal testing, biofilms (in 96-well plates) were challenged with amphotericin B (0.06 - 16 mg/l), caspofungin (0.008 to 2 mg/l), and fluconazole (4 - 1024 mg/l) alone or in combination with DNase (0.125 mg/ml) or exogenous DNA (320 ng/ml). Sessile minimum inhibitory concentrations (SMIC) were determined at 80 % inhibition compared to drug-free controls using the XTT reduction assay. RPMI medium was used in all the assays.

On one hand, *C. albicans* biofilms ECM contained 3045.4 ± 227.3 ng eDNA/mg of protein. On the other hand, DNase or exogenous DNA treatments did not affect further biofilm development by *C. albicans* adherent cells. In contrast, DNase (> 0.03 mg/ml) promoted a general biomass reduction on *C. albicans* preformed biofilms, as indicated by the reduction of $A_{550}$ compared with the control. Furthermore addition of exogenous DNA (> 160 ng/ml) to preformed biofilms led to an increase in biofilm biomass, similarly assessed by the higher $A_{550}$ readings compared with control biofilms. Finally, DNase I (0.125 mg/ml) did not change *C. albicans* biofilm cells susceptibility to fluconazole, but increased their susceptibility to amphotericin B and caspofungin, as indicated by the lower SMIC compared to biofilms grown without DNase. In contrast, exogenous DNA (320 ng/ml) did not affect *C. albicans* biofilm cells susceptibility against these antifungals.

This work presents evidence for the role of eDNA in *C. albicans* biofilm integrity and antifungal resistance consistent with eDNA being a key element of the ECM in *C. albicans*.