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TITLE

Role of extracellular DNA in *Candida albicans* biofilms

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ABSTRACT

DNA has been described as a structural component of the extracellular matrix in bacterial biofilms. However, in *Candida albicans* there is a scarce knowledge concerning the contribution of extracellular DNA (ecDNA) to biofilm matrix and overall structure.
The main objective of this work was to examine the effect of Deoxyribonuclease I (DNase) treatment and the addition of exogenous DNA on *C. albicans* biofilm as indicators of the role of ecDNA in biofilm structure and development.
Using a rapid and robust method (96-well plate model), the effect of DNase (ranging from 0.02 to 2 mg/ml) and exogenous DNA (ranging from 10 to 2560 ng/ml) on biofilm formation was examined at different stages of *C. albicans* biofilm development. Specifically, standardized cell suspensions (100 µl of 1×10^6 cells/ml in RPMI) were inoculated into 96-well microtitre plates and incubated for 0, 1, 2 and 24 h at 37°C under static conditions. At 0-h (preincubation), DNase or exogenous DNA were added to the standardized suspension. At 1-, 2- and 24-h of incubation the medium was removed and adhered cells were washed with PBS. DNase or exogenous DNA working solutions were then added to *C. albicans* sessile cells. The plates were incubated at 37 °C for additional 24 h. Biofilm biomass was evaluated by crystal violet assay.
The results of these experiments showed that DNase did not exhibit major effects on *C. albicans* adherent cells at early time points of biofilm development. However, DNase showed a general biomass reduction on *C. albicans* preformed biofilms, with the reduction of absorbance at 550 nm compared with the control at DNase concentrations higher than 0.03 mg/ml. The addition of exogenous DNA to *C. albicans* adhered cell populations did not affect further biofilm development. In contrast, addition of exogenous DNA, at concentrations higher than 160 ng/ml to preformed biofilms led to an increase in biofilm biomass, as indicated by the higher levels of crystal violet readings compared with control biofilms.
Thus, we present evidence of the role of ecDNA in *C. albicans* biofilm structure, consistent with ecDNA being a key element of the ECM in mature *C. albicans* biofilms and playing a predominant role in biofilm structural integrity and maintenance.