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THE ABILITY OF *C. ALBICANS* TO FORM BIOFILMS IN THE PRESENCE OF SENSITIVE AND PHAGE-RESISTANT *P. AERUGINOSA* PHENOTYPES

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P. aeruginosa and *C. albicans* are human opportunistic pathogens frequently associated with nosocomial infections. Pathogenic interactions between them have already been identified and it has been reported that *C. albicans*' morphology and virulence are significantly affected by the presence of *P. aeruginosa*. In this work, the interaction between these 2 microorganisms in mixed biofilms was studied. The behavior of *C. albicans* cells after applying *P. aeruginosa*-specific phages, which lyse *P. aeruginosa* biofilm cells in the mixed biofilms, was analyzed as well as the ability of *C. albicans* biofilm formation in the presence of *P. aeruginosa* phage-resistant phenotypes. Biofilms were formed in 24-well microplates, containing 1 ml of YPD medium and 10 μ l of each cellular suspension ($OD_{600nm} = 1$), during 24 and 48 h. Phages (phiIBB-PAA2 and phiIBB-PAP21) were applied in 24 h old mixed biofilms and samples were taken after 2, 6 and 24 h of phage biofilm infection for CFU counts.

The results revealed that *C. albicans* proliferation was clearly inhibited by the presence of wildtype *P. aeruginosa* ATCC 10145 and PAO1 strains. Conversely, the proliferation of *P. aeruginosa* was not influenced by the presence of *C. albicans*. After the infection, it was observed that both phages were effective in depleting *Pseudomonas* biofilm cells from

the mixed biofilms. This reduction resulted in an increase in the amount of *C. albicans* cells during phage infection. However, at 24 h post-infection of mixed biofilms, an increase of *P. aeruginosa* biofilm cells was also observed as compared to the numbers at 6 h post-infection. This suggests that *P. aeruginosa* cells acquired resistance to the phages between 6 and 24 h of infection. Surprisingly, this increase in *P. aeruginosa* at 24 h post-infection did not interfere with *C. albicans* biofilm growth and accordingly, an increase in *C. albicans* cells was observed. These results suggest that the surviving *P. aeruginosa* cells after phage attack have changes in their phenotype resulting in a diminished ability to inhibit *C. albicans* biofilm growth.

To evaluate if the regrowth of *C. albicans* cells in the infected biofilms was caused by the emergence of phage-resistant *P. aeruginosa* phenotypes, which did not inhibit *C. albicans* biofilm growth, several *P. aeruginosa* colonies were isolated after 24 h of mixed biofilm infection with phage phiIBB-PAA2 and tested for their susceptibility to the phages used in this study. Most of these phenotypes showed resistance to the phage phiIBB-PAA2 and 1 of these strains was also resistant to phage phiIBB-PAP21. Mixed biofilms with *C. albicans* were once again performed with these *P. aeruginosa* ATCC 10145-phenotype variants and revealed that most of them could co-inhabit better with *C. albicans* than their wildtype strain.

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