

ABSTRACT

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Title of thesis: Optimization of methods for sensitivity evaluation of biofilm-forming microbial agents towards substances with antimicrobial potential

Background: The aim of this diploma thesis was to optimize the methodical approach to the production of yeast biofilms in vitro on the so-called pegs and the walls of the wells of the 96-well panel. This biofilm formation system should be an analogous approach to the commercially available Calgary Biofilm Device. 10 strains of *Candida* yeast and 4 different culture media (nutritionally poor / rich) were used to optimize the methodology. Both the reference strains and the clinical isolates were among the yeast strains tested.

Methods: The ability to form yeast biofilms by different strains in different culture media was evaluated by the approach of fixation, staining of the formed biofilms by crystal violet and extraction and spectrophotometric measurement of the intensity of the extracted color.

Results: Individual strains, after cultivation in different media, were categorized according to their ability to form biofilm. Larger yeast adherence occurs in the wells than on pegs where the yeast adhered very weakly or at all. Most *Candida albicans* strains produced a larger biofilm mass in media containing fetal bovine serum, i.e. in MGM10 and RPMI media. However, in the case of *Candida tropicalis*, there was more than 10-fold higher adherence to the bottom and wells of the plate in Soyabean medium.

Conclusion: The ability to adhere to the polypropylene pins has not been demonstrated in our yeast strains. Fetal bovine serum, based on statistical evaluation, demonstrably supported the growth and formation of biofilms in most yeast *Candida albicans* strains in microtiter plate wells. The *Candida tropicalis* yeast strain tested in the Soyabean medium proved to be a strong biofilm producer and will be used to further optimize the methodology.

Key words: biofilm, in vitro testing of microbial sensitivity, physiology of biofilms, *quorum sensing* phenomenon