The Effects of Estrogen (E2) on the Filamentous Growth of *Candida albicans* on Assorted Solid Medias

Paige M. Camp*. George Gundelach*, Idalia Zachara, Anita Coleman, Riquail Gibson, Briana Williams, Micheal K. Watters and Patrice G. Bouyer
(* equally contributed author)
Biology Department, Valparaiso University, IN USA

ABSTRACT

Candida albicans is a commensal yeast that lives within the human body. When exposed to specific environmental stimuli (ex. pH, temperature) the fungi will undergo a morphological change from yeast to filamentous (hyphal). Currently, *C. albicans* is the most prevalent human fungal pathogen, causing both mucosal and systemic infections. This infection, called candidiasis, can endanger high-risk groups, such as immunocompromised patients. While studied extensively, the relationship between *C. albicans* hyphal growth and estrogen (E2) has yielded mixed results amongst researchers. In our research, we attempted to provide clarity to this relationship by using a variety of solid media and studying how each affects E2's influence on hyphal development. We used three solid media; Minimum, Spider, and YEPD. In our experiments, we found that *C. albicans* undergoes three distinct morphologies and variable behavior between the three media types. In Minimum media, we found that E2 is blocking filamentous growth after day two compared to no E2. In Spider media, E2 is blocking filamentous growth up to day three compared to no E2. Finally, in YEPD, E2 inhibits filamentous growth only at day two compared to no E2. In our conditions, E2 has an inhibitory effect on filamentous growth. Furthermore, our observations suggest that the inhibition is media-dependent. Currently, we are testing fetal bovine serum (FBS), which is known to induce filamentation, as a positive control to compare to the effects of E2.

INTRODUCTION

Candida albicans is a yeast that lives naturally in the human gut. When exposed to certain stimuli, such as changes in pH or temperature, *C. albicans* can transition from a harmless yeast morphology to a virulent (disease-causing) filamentous morphology. These filaments can be used to "drill" into healthy host cells, causing extensive tissue damage. This infection of virulent *C. albicans* is known as candidiasis. The gut cues responsible for the transition are not well characterized.

17- β estradiol (E2) AKA as estrogen is a steroid hormone that plays a large role in female reproductive cycles and the formation of secondary sexual characteristics. In cases of severe sepsis, E2 is secreted much higher concentrations [1]. As systemic *candida* infections are prevalent in critically ill patients, we hypothesized that E2 might be a cue responsible for *C. albicans* morphological changes.

MATERIALS AND METHODS

Strain and culture media: In this study, we used the fungal *Candida albicans* (*C. albicans*) and three different solid agar media: Minimum media (MM), Spider and YEPD. Agar plates were inoculated with *C. albicans* using an inoculating loop and plates were incubated at 30˚C in an air incubator (see figure 1).

Estrogen (E2) and fetal bovine serum (FBS): To test the effect of E2 and FBS on filamentous growth, 350 µl of a 0.1 nM E2 solution or 350 µl of FBS was spread onto plates. When testing the combination of E2 and FBS 160 µl of each solution was spread onto plates prior inoculating plates with *C. albicans*.

In a separate experimental series, we have have started to add E2 (0.1 nM) and FBS 10% V/Vol to the liquid agar before pouring plates to match the condition in liquid media (see other poster). In these conditions, plates are inoculated with approximately 5,000 yeast cell grown in liquid-MM.

Morphology assessment: Morphological changes were visualized using bright-field microscopy. Images were captured using a Leica DM4B microscope equipped with a MC170 HD camera driven by the Leica Acquire software. *C. albicans* morphology images were captured using a 10x 0.25 NA objective.

Figure 1: Example of agar plates inoculated with *C. albicans* and grown at 30°C in an air incubator.

RESULTS

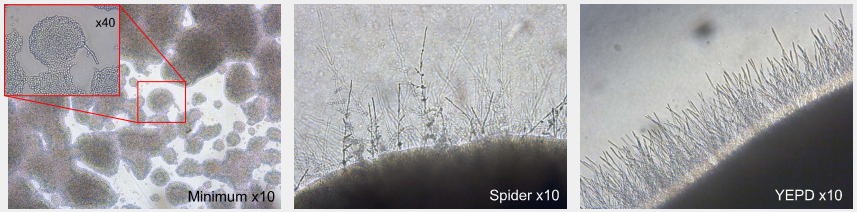

Figure 2: Representative morphology of *C. albicans* on the three solid media used in the study.

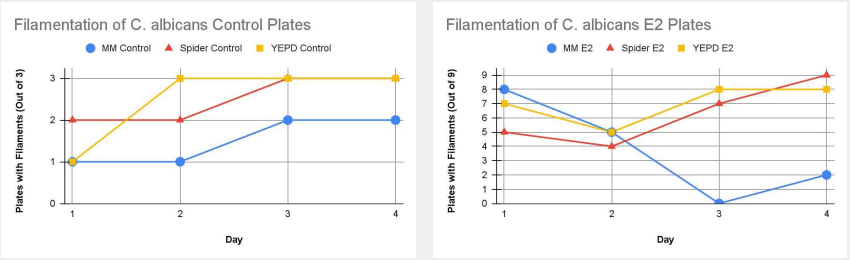

Figure 3: Time course of filaments growth over time. On the left panel (control), filament growth was observed every day during a four day period. In this experimental series, three control plates were used. As shown on the graph, filamentous growth was observed in all three control media. The right panel, displays a similar time course but in presence of E2. In MM E2 strongly inhibited filamentous growth over the 4 day time span. However, involving the Spider and YEPD media, E2 inhibition persisted only one day.

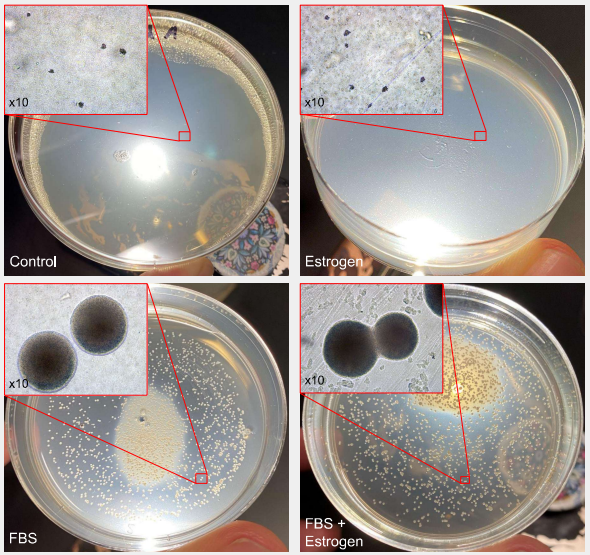

Figure 4: Effect of E2 and fetal bovine serum (FBS) on filamentous growth. In these preliminary experiments E2 (0.1 nM), and FBS (10% v/vol) were directly embedded in the liquid agar at the time the plates were poured. As shown on the pictures, at day 1, *albicans* colonies were very discreet in control and E2 plates, but a striking growth of colonies was observed on FBS and FBS+E2. However, looking at a higher magnification (insert pictures on the left corner), we did not see in none of our current experimental conditions filamentous growth.

CONCLUSIONS

C. albicans morphology

- In our experimental conditions, we observed that *C.albicans* displayed a distinctive morphology depending on the solid media it is grown on (see figure 2), which is probably caused by the composition of the medium.

Effect of E2 on C. albicans morphology

- During the time lapse experiments (figure 3), we observed that E2 effectively inhibits filamentous growth during the 4 days time course.
- Alternatively, E2 inhibition of filaments growth is observed during the first 2 days of culture of *C. albicans* in spider and YEPD media.
- In our current experimental conditions, we conclude that the inhibitory effect of E2 on filament growth is media dependent.

Effect of FBS and E2 embedded in plates

- In the preliminary experiments (see figure 4), we observed that FBS significantly increased colony growth compared to control and E2.

FUTURE PLANS

Future Direction:

- Because the concentration of E2 and FBS may vary across plates on which we spread liquid E2 and FBS, we are moving to the approach described in figure 4.
- Our current results produced more colonies than we were expecting, so we are doing more tests at different concentrations of cells to find the optimal amount of colonies.
- In contrast to FBS, which is known to promote filamentation, we would like to test the effect of farnesol, a molecule known to block filamentous growth, to compare the effect of E2 to a known blocker of filamentous growth.
- Based on some of our current results, we are exploring the idea of doing an automated time lapse using another microscope that will allow us to take pictures of the same spot at any time interval we decide on.
- To validate the results and conclusions that have been produced, it will be an utmost priority to replicate this experiment.

ACKNOWLEDGEMENTS

The authors would like to thank the Indiana Space Grant Consortium for their financial support during the course of the study. Valparaiso University for the opportunity to do a summer research project. The authors also would like to acknowledge and thanks the organizers of the the summer activities. Finally, a special thank for our mentors Prof. Bouyer and Prof. Watters.

Reference

1. **Lesly A. Dossett *et al.* High Levels of Endogenous Estrogens are Associated With Death in the Critically Injured Adult. *J Trauma* Chem. 2008, 64 (3): 580-585**
2. **D.W.R. Mackenzie *et al.* Serum Tube Identification of *Candida albicans*. J Clin Pathol. 1962, 3**
3. **Addison K. May *et al.* Estradiol is associated with mortality in critically ill trauma and surgery patients. NIH-PA. 2008, 15**
4. **S. White and B Larson. *Candida albicans* morphogenesis is influenced by estrogen. CMLS. 1997, 6**
5. **S. Kurakado *et al.* 17β-Estradiol inhibits estrogen binding protein-mediated hypha formation in *Candida albicans*. Elsevier Microbial Patho. 2017, 5**