Effects of Environmental Factors on *Candida albicans* Morphology: A Focus On Estrogen and Microgravity

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ABSTRACT

During body invasion, *C.albicans* change their morphology from yeast to filamentous, but the environmental factors responsible for the change in morphology are not well characterized. During Sepsis, high levels of estrogen (E2) are recorded (~0.1 nM), in addition during spaceflight it has been shown that *C.albicans* become virulent. This study aimed at characterizing the effect of estrogen and microgravity as environmental factors inducing filamentous growth. *C.albicans* was grown in minimum liquid media and brightfield microscopy was used to observe its morphology. Microgravity was simulated using a clinostat. In an experimental series, the effect of FBS (positive control) and estrogen were tested on filamentous growth. It was found that in the control only 1 out of 10 slides showed filaments. In the presence of FBS, filamentous growth was observed in 10 out of 10 slides. In the presence of estrogen, filaments were seen in 8 out of 10 slides. In addition to FBS, the combination of FBS + E2 showed filamentous growth in 10 out 10 of the slides. However, in the presence of microgravity, filaments were observed in 9 out of 10 slides, meanwhile, only 4 out of 10 slides without microgravity had filamentation. In addition to microgravity, the combination of E2 + Microgravity, filaments were observed in 3 out of 10 slides. Meanwhile, only 4 out of 10 slides with just E2 exhibited filaments. In conclusion, estrogen does not inhibit filamentous growth stimulated by FBS, but it prevents filamentous growth in microgravity.

INTRODUCTION

Candida albicans is a fungal that lives naturally in the human gut, that can shift its morphology from yeast to filamentous (see figure 1) when exposed to an increase of temperature or alkaline pH. In its filamentous form, *C. albicans* can invade epithelial cells. The filament attaches to cell and can start perforating the epithelial layer causing extensive tissue damage.

The hormone estrogen (E2) 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 than normal and can reach concentration of 0. 1 nM (Dossett, 2008). In addition, during severe sepsis, systemic *C. albicans* infection is common. To date, the environmental factors responsible for the shift in morphology are not well characterized. In the present study, we hypothesized that a raise of E2 may be responsible for the morphological changes in *C. albicans.*

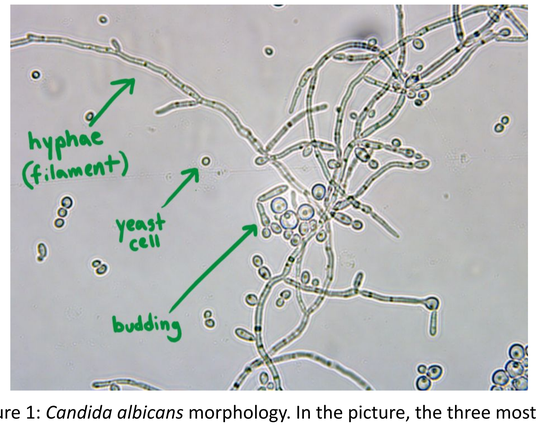
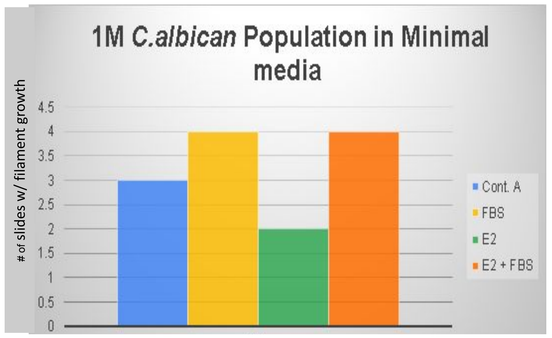


Figure 1: *Candida albicans* morphology. In the picture, the three most common morphologies of *C. albicans* are represented: Yeast cell, the budding yeast (AKA pseudohyphae) and filamentous or hyphae.

RESULTS

Effect of Estrogen and fetal bovine serum on filamentous growth in *C. albicans*



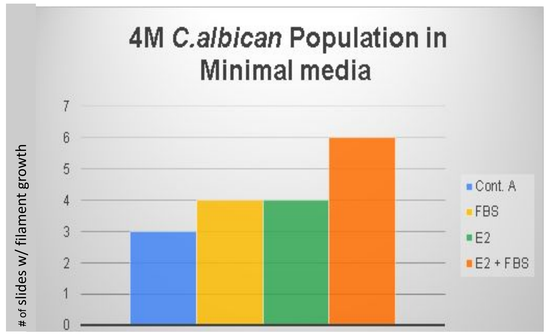
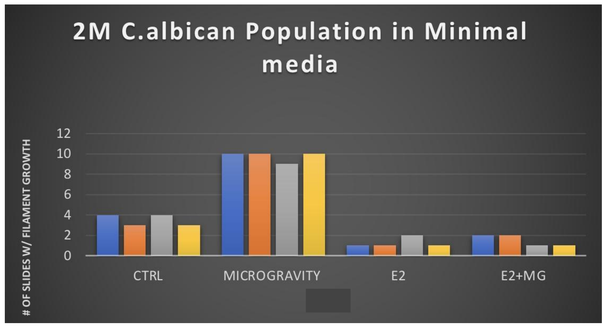


Figure 2: In this experimental series, we tested the effect of fetal bovine serum (FBS) and estrogen (E2) of the filamentous growth after two days culture in minimum liquid media. In presence of FBS (positive control to induce filamentous growth) an increase of the number of slides showing filamentation was observed in *C albicans* cultured at 1 million (M), left panel, and 4 M (right panel). On the other hand, E2 decreased the number of slides with filamentation in the 1 M group, but increased the number of observation of slides showing filamentous growth in the 4 M group. Combining FBS and E2 resulted in the same number of observation of slides with filaments than in FBS alone in the 1 M group. Alternatively, an synergistic effect of FBS and E2 on the number of slides showing filaments was observed in the 4 M group (right panel).

Effect of Microgravity and Estrogen on filamentous growth in *C. albicans*



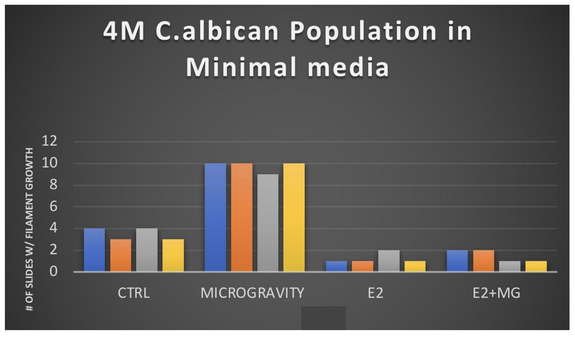
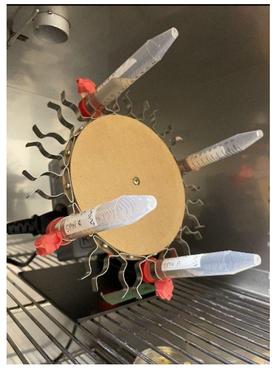


Figure 3: In this experimental series, the effects of microgravity and E2 on filamentous growth were tested after three days in cultured minimum media. In the presence of microgravity, an increase of the number of slides showing filamentous growth was observed in *C. albicans* cultured at 2 million (M), left panel, and 4 M (right panel) compared to the control (CTRL). Exposing *C. albicans* to 0.1 nM E2 decreased number of slides with filamentation in both group 2 M and 4 M compared to CTRL. Finally, combining microgravity and E2 resulted in an inhibition of the filamentation induced by microgravity. Each colored bar represents an experimental trial

Figure 4: microgravity simulating apparatus. Left and right panels show a clinostat holding tubes. The clinostat completes a full rotation in 1 hour, and the shear stress produced during the rotation mimics microgravity. On the left side panel, a rack containing tubes is shown at the bottom of the incubator. The tubes placed in this rack serve as control, no microgravity.



MATERIALS AND METHODS

For these experiments *C.albicans* was grown in minimum liquid media (MM) and brightfield microscopy was utilized to observe the morphology. The number of cells per milliliter was estimated using a spectrophotometry approach as previously described by Morris and Nicholls (1978). The following correlation between the number of cell and absorbance was found for minimal media $Y = 1.06 \times 10^7 + 1.83 \times (OD) + 1.68 \times 10^7(OD)$. 1 and 4 million cell total cells were utilized in our experiments. E2 was used at a concentration of 0.1 nM to mimic the systemic level of E2 observed in patients with sepsis (Dossett, 2008). Fetal bovine serum (FBS), a factor known to induce filamentation in *C. albicans* was used as a positive control in the experiments (Mackenzie, 1962). In the present study, a 10% V/V FBS was used to induce filamentation.

Minimum media was inoculated with *C. albicans* and placed in a shaking incubator at 30°C overnight. The number of cells was estimated as described above and tubes containing 1 million and 4 million C. *albicans* were prepared. For microgravity experiments, a clinostat (see figure 4) was used to simulate microgravity. Microgravity subjected tubes and controls tubes were placed within an incubator at 30°C (see figure 4).

A DM4-B Leica microscope equipped with a 10x objective and MC170 HD camera was used to capture images.

CONCLUSIONS

C. albicans and FBS

- Culturing *C.albicans* in minimum liquid media in presence of FBS yielded to more filamentations than in control group

C. albicans and Estrogen

- E2 yielded less filamentations than the control and FBS group in the 1M goup, on the other hand, E2 yielded to the same amount of slides with filaments than FBS in the 4 M goup (Figure 2).

C. albicans and Combination of E2 + FBS

- Exposing *C.albicans* to both E2+ FBS yielded to similar number of slides with filaments in the 1 M goup compared to FBS alone. Contrastingly exposure of *C. albicans* to E2 and FBS yielded to the highest amounts of filamentation on slides in the 4M group.

C. albicans and Microgravity

- *C. albicans* exhibited an induced filamentation effect over the course of three days within minimal media when exposed to microgravity compared to control.

C. albicans and Estrogen

- E2 yielded less filamentations than the control or microgravity group

C. albicans and Combination of Microgravity + E2

- In *C.albicans* subjected to microgravity and E2 a significant decrease of filamentation was observed compared to microgravity alone.

Future Direction:

- Test the effect of estrogen and FBS and microgravity on other media (YEPD and spider).
- Investigate the cellular mechanism of filamentation during microgravity and the signaling pathway of E2

ACKNOWLEDGEMENTS

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