

ABSTRACT

C. albicans is a commensal fungus which under certain environmental cues shifts morphology from spores to filamentous and becomes invasive within the human body. During sepsis, blood estrogen (E2) becomes elevated. E2 is also known to promote tissue growth, thus we hypothesize that E2 may influence *C. albicans* growth. In the present work, we investigated the effect of estrogen on *C. albicans* colony sizes grown on yeast extract peptone dextrose (YEPD). Using bright field microscopy, images of five colonies in each condition were captured on day one and pictures of the same colonies were captured on day two. The diameter of each colony was computed using ImageJ and the surface area was calculated using Excel. On day one, one-way ANOVA shows no difference ($P=0.09$) in colony size for the 4 conditions tested (control 0.19 ± 0.09 mm², 0.1nM E2 0.20 ± 0.08 , fetal bovine serum (FBS) 0.23 ± 0.11 , FBS+ E2 0.24 ± 0.12). On the second day, the size of each colony significantly increased compared to day one ($P<0.001$, paired t-test) for each individual condition (i.e., control day 1 versus control day 2). Comparing colony size increase (surface area on day 2- surface area day1) a significant difference among the 4 conditions $P<0.001$, one-way anova. dunnett post hoc test shows no significant difference between control 2.41 ± 0.29 mm² vs E2 2.46 ± 0.35 ($P=0.8$) but a significant difference between control 2.41 ± 0.29 versus FBS 2.69 ± 0.27 ($P<0.001$) and control 2.41 ± 0.29 vs FBS+E2 2.71 ± 0.35 ($P<0.001$). In conclusion, our results show that fbs significantly increased colony growth in YEPD, but E2 had no significant effect on colony growth.

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 (spore) to a virulent (disease-causing) filamentous morphology. These filaments can be used to "drill" into healthy host cells, causing extensive tissue damage [1]. 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 [2]. As systemic candida infections are prevalent in critically ill patients, we hypothesized that E2 might be an intestinal cue that affect *C. albicans* growth rate.

MATERIALS AND METHODS

Strain and culture media: In this study, we used the fungal *Candida albicans* and two different solid agar media: Spider and YEPD. *C. albicans* was grown overnight in liquid media that corresponds to the solid media it was destined for and we used approximately 300 *C. albicans* to inoculate agar plates

Infused agar plates: To test the effect of E2 and FBS on colony growth, E2 and FBS were mixed into the molten agar to concentrations of 0.1 nM E2 or 10% V/Vol FBS. When testing the combination of E2 and FBS, the plates contained both 0.1 nM E2 and 10% Fetal Bovine Serum (FBS).

Identification of colonies: Plates were allowed to grow at 30°C in an air incubator overnight. On Day 1, colonies were identified using a dissecting microscope and circled with a marker on the underside of the agar plate. On Day 2, the same colonies were tracked. Colony growth was observed 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* colony images were captured using either a x5 or a 10x 0.25 NA objective.

Analysis of images: Images captured were processed using Fiji (equivalent to imageJ). A custom macro was developed to ensure that all images were processed using the same procedure. This macro was able to identify, outline, and measure the colonies to a high degree of accuracy as shown in figure 1. The macro then measured the diameter and results were exported to an excel file for calculation of the surface area.

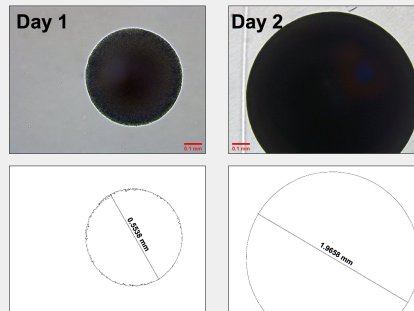


Figure 1: Example of the macro from Fiji measuring colony diameter

RESULTS

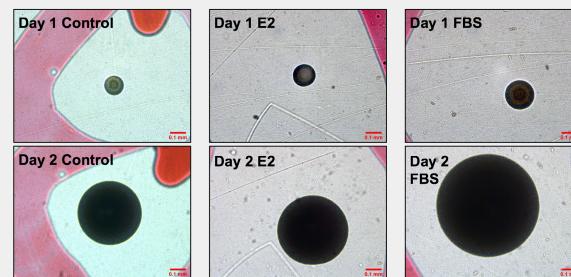


Figure 2: *C. albicans* colonies growth over two days period in Spider in control, presence of E2 and FBS

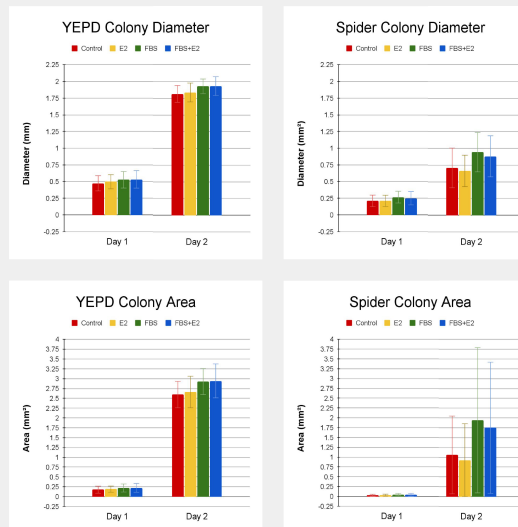


Figure 3: Colony diameter and surface area in YEPD and Spider media in control, and in the presence of 0.1 nM E2, 10% v/vol FBS and combination of E2 and FBS

Condition	YEPD P value	Spider P value	Combined P value
E2	0.4	0.6	0.9
FBS	<0.001	0.006	<0.001
Day	<0.001	<0.001	<0.001
E2-FBS	0.6	0.8	0.7
YEPD	NA	NA	<0.001

Table 1: Mixed effect model statistical analysis. As shown in the table E2 had no effect on colony surface area compared to control. FBS significantly increased surface area compared to control. E2 + FBS had no significant effect compared to control. A significant increase in surface area was observed between day 1 and 2. Finally, the mixed effect model revealed that surface area was 0.76 mm² bigger in YEPD compared to Spider and it was statistically significant.

CONCLUSIONS

Estrogen has been reported to influence growth of *C. albicans* at concentration of 1 μ M [3], which is above the pathological range observed in sepsis [2]. In this study, we investigated the effect of E2, FBS and FBS + E2 on *C. albicans* growth in two solid media YEPD and Spider. In our conditions, we found:

- FBS significantly increased colony growth in both media compared to control
- E2 did not significantly increase the size of the colony compared to control
- No interaction between FBS and E2 was found on the growth of the colony
- Colonies grown on YEPD medium were 0.76 mm² bigger than the one grown on Spider medium

Our study contrast with others who found increase of growth of *C. albicans* but using higher concentration of E2. Further investigations are needed to test whether E2 affects *C. albicans* growth in the host.

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