

Testing Environmental Cues on Candida albicans Morphology Idalia Z. Zachara*, Paige M. Camp*, Michael K. Watters and Patrice G. Bouyer (* equally contributed author) Biology Department, Valparaiso University, IN USA



Introduction

C. albicans is a commensal fungus which under certain environmental cues shifts morphology from spores to filamentous and becomes invasive within the human body. Ambient CO₂, pH, and oxidative stress have been shown to cause morphological changes in C. albicans. This work aims to identify the environmental gut cues responsible for this morphological shift. Estrogen (E2) becomes elevated during sepsis, thus the guiding hypothesis states that E2 may represent a factor responsible for the morphological change in C. albicans.

Methods

A calibration curve of growth of *C. albicans* in liquid minimal media (MM) was established using a spectrophotometer and correlating optical density with cell counts measured with a hematocyter. C. albicans was inoculated in MM in amounts of 1, 2, and 4 million cells into sets of 4 tubes each based on the established growth curve. To test the effect of estrogen at 1nM concentration, E2 was added at the time of inoculation to one of each tube set, and 10% fetal bovine serum (FBS) was the positive control in another tube. All tubes were anaerobically grown over 3 nights in a shaking incubator at 30°C. Morphological changes were assayed using bright-field microscopy. Images were captured using a Leica DM4B microscope and Leica Acquire software, using a $40 \times$ objective.



Figure 1. Row A shows experiments done with 1 million C. albicans, while B shows 2 million and C shows 4 million. Column 1 represents C. concentrations would bring more insight, as well as trials albicans in plain MM, column 2 in presence of E2, and column 3 in presence of 10% FBS.

Results

MM was inoculated with 1, 2, and 4 million C. albicans into sets of 4 tubes each based on the established growth curve (graph 1). The MM relationship between OD and number of spore cells is described by the following equation: $1.06 \times 10^6 + 1.83 \times 10^7 x + 1.68 \times 10^7 x^2$, R²= 0.867. Adding E2 at 1 nM to the liquid media (fig. 2A-C) appeared to induce filamentous growth and budding, as shown in positive control 10% FBS (fig. 3A-C).



Graph 1. Calibration curve of growth of *C. albicans* in liquid MM.

Conclusion

Our preliminary experiments indicate that regardless of initial cell amount, tubes containing E2 seem to induce filamentous growth in MM as observed with FBS (positive control). Further experiments to determine effects of E2 at other combining E2 and FBS to explore if there is an additive or inhibitory effect on filamentation.

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