Environmental Cues and Fungi Morphology

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ABSTRACT

Candida albicans, a symbiotic yeast in the human gut, and ***Neurospora crassa***, a filamentous bread mold, are distinct in habitat, morphology, and behavior. However, both fungi are equally susceptible to the ongoing flow of stimuli present within the environment. The objective of our research is to understand how different fungi respond to specific cues found within or outside their natural environment. Four stimuli were tested on *C. albicans*: estradiol (E2), media morphology, and irradiated and non-radiated plastic microfibers. Three stimuli were tested on *N. crassa*: plastic non-radiated microfibers, simulated microgravity, and cold shock. *C. albicans* was tested only on solid agar plates, while *N. crassa* was tested on both liquid and solid agar media. Specialized minimal media plates containing microfibers were made to test irradiated and non-radiated microfiber exposure. While C. *albicans* expressed no sensitivity to 0.1nM E2, it displayed three types of morphology when grown on either minimal, Spider, or YEPD media. *N. crassa* showed no sensitivity towards microfibers, but *C. albicans* exhibited varying degrees of inhibition for colony formation. Under simulated microgravity, *N. crassa* did not show significant morphological differences besides a possible increase in the amount of conidia present, however, results are inconclusive.

INTRODUCTION

Candida albicans is a fungus that lives commensally within the human gut. Typically a harmless yeast, *C. albicans* has been recorded to become virulent when exposed to certain environmental cues (pH, temperature, etc). Virulent *C. albicans* undergo a morphological change into a filamentous fungus, using these filaments to drill into healthy cells and causing an infection known as candidiasis. *C. albicans*-associated candidiasis can cause severe complications in high-risk demographics such as elderly and immunocompromised patients.

Neurospora crassa is a filamentous bread mold that is commonly used as a model organism for fungal and genetic research. *N. crassa* exhibits three phases of an asexual life cycle: conidial germination, vegetative growth, and mature conidiation. Starting with conidial germination, individual cells go through internal changes within the cell wall to form and elongate their germ tube. From there, vegetative mycelial growth occurs, segmenting and foming hyphal tips rapidly. Finally, mature conidia appear from newly formed vegetative mycelia through four stages. Due to the high amount of *N. crassa* that appeared on slides, our research focused on the last two stages, septation and maturation. Conidia septation includes division between each cell, allowing conidia to seperate, while maturation is the ~3 day process of conidia forming their own germ tube and branching off.

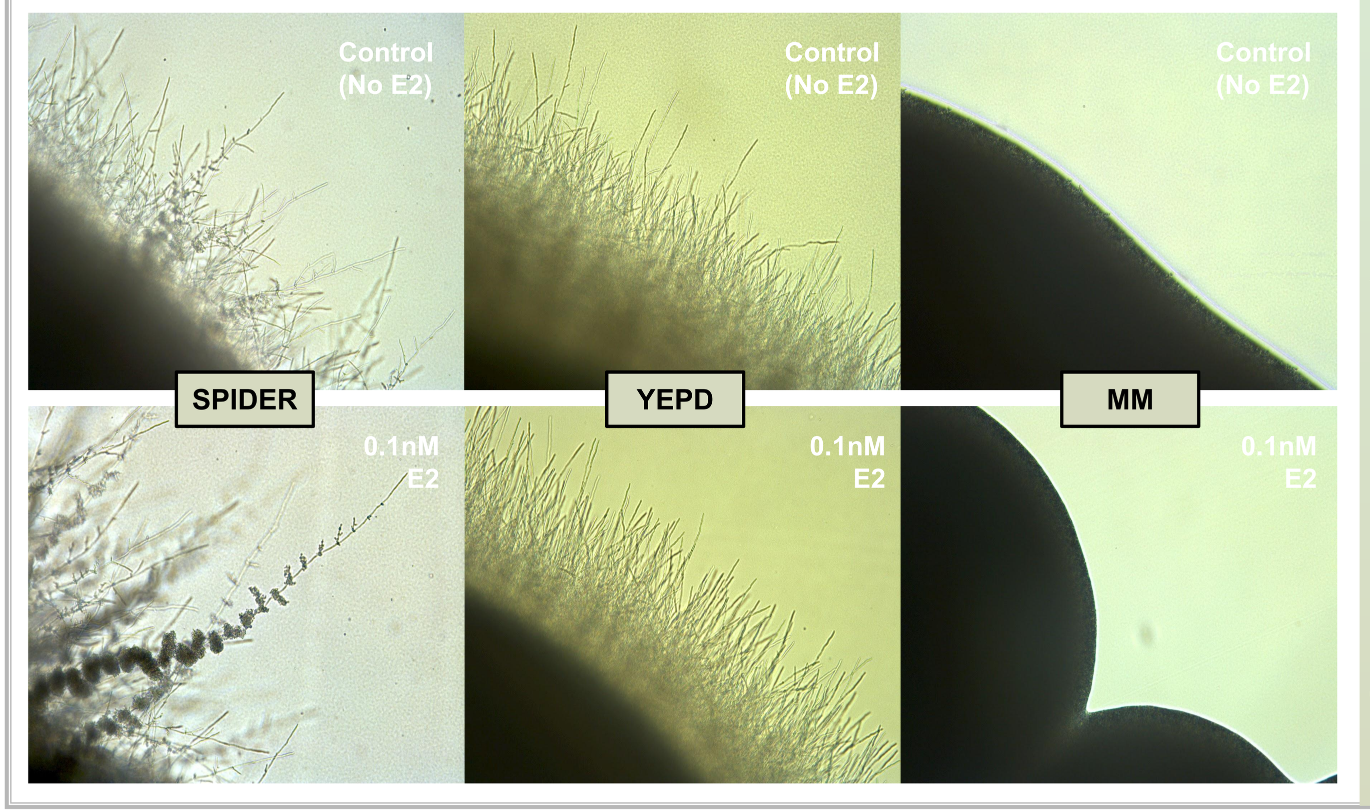
Higher estradiol concentrations (0.1nM E2) within the human body have been recorded alongside life-threatening sepsis, as well as the emergence of virulent *C. albicans* and, subsequently, candidiasis. This correlation has led to questions on the impact of estrogen on *C. albicans* morphology.

Microfibers are synthetic fibers that have diameter of less than ten micrometers. These plastic fibers can either be shed from damage or discarded clothing or released during washing, which can cause pollution freshwater and soil. Microfibers are a source of interest for fungal morphology research due to their prevalence within the environment. Particularly, freshwater microfiber pollution has been observed to alter the microbiota within the gut of zebrafish, causing intense inflammation. Since *C. albicans* is commonly found in the human intestinal microflora, the interactions between ingested microfibers and the yeast is a cause for concern.

The study of *N. crassa*'s growth in simulated microgravity is important not only because as a model organism *N. crassa* can help show us understand how cellular processes and fungal development in general are affected by microgravity, but also because the understanding of how molds in particular respond to microgravity. Molds and other fungi can pose a high risk within spacecraft, as they may cause biodegradation to the craft or systems within, and as the molds found in spacecraft are shown to have a negative impact on the immune system.

RESULTS

Estradiol (E2) and Media Morphology

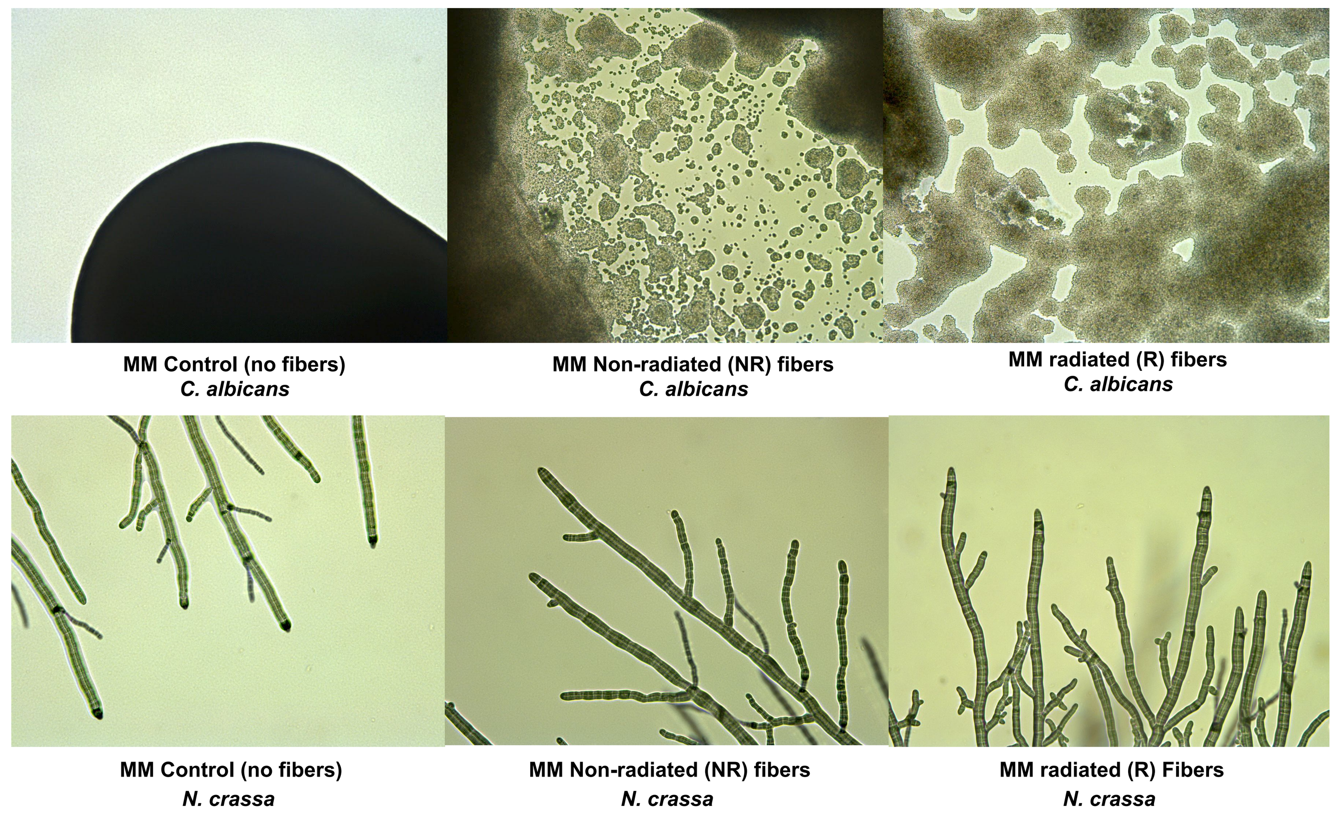


C. albicans grown on Spider media consistently exhibited morphology **type B** with and without 0.1nM E2.

C. albicans grown on YEPD media consistently exhibited morphology **type C** with and without 0.1nM E2.

C. albicans grown on MM media consistently exhibited morphology **type A** with and without 0.1nM E2.

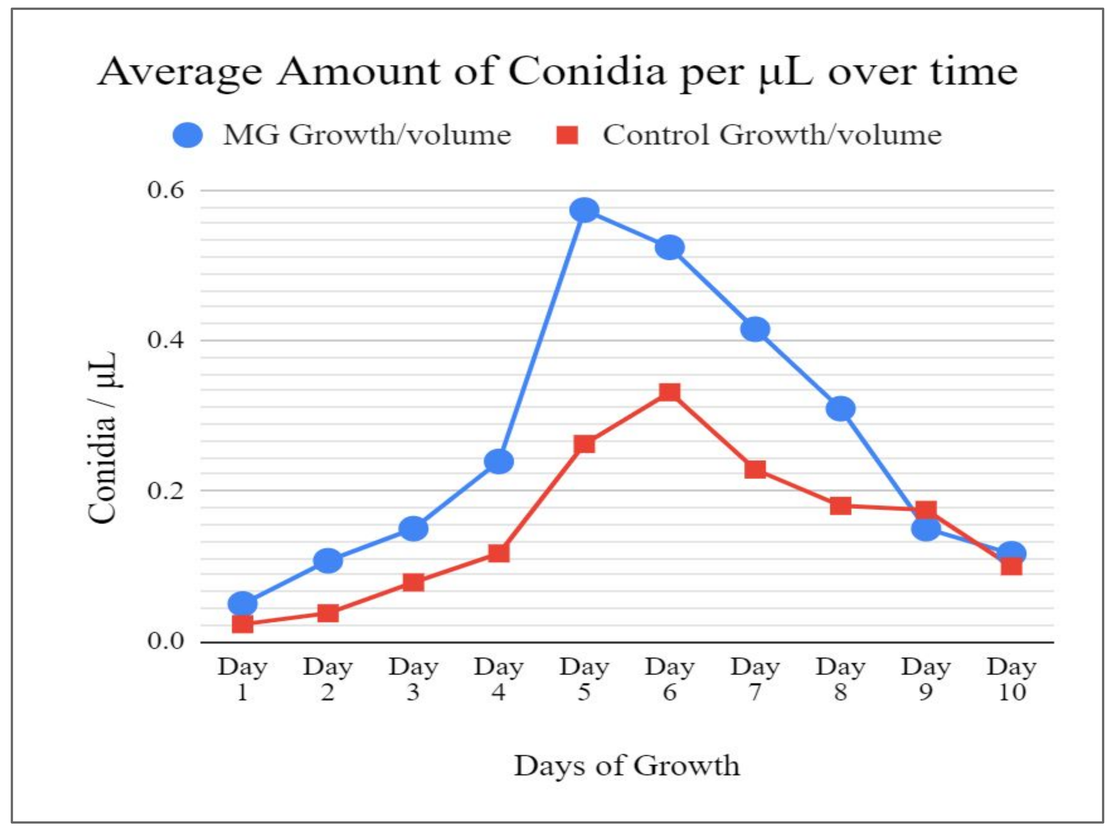
Growth in Plates with Microfibers



C. albicans grown in microfiber MM plates experienced varying levels of inhibition in colony formation. Smaller colones in NR microfiber plates appeared more fragmented than those grown in R microfiber plates.

However, *N. crassa* showed no notable change between the three media plates.

Simulated Microgravity and Conidia Growth



N. crassa grown in MM tubes without simulated microgravity exhibited normal growth with normal production of conidia at all four stages.

N. crassa grown in MM tubes that were subjected to simulated microgravity exhibited an increase in conidiation per volume of tube, as well as an increase of individual germination on slides.

On average, the microgravity slides contained 1.83 times as many conidia as those in the control.

MATERIALS AND METHODS

For the *C. albicans* research, plates with solid Minimal Media (MM), YEPD media, and Spider media were used, and for the microgravity research with *N. crassa* test tubes with 10mL of liquid MM were used.

C. albicans filament morphologies were categorized as A, B, and C. With A representing smooth-edged colonies, B representing "tree-like" filaments, and C representing "grass-like" filaments. For each plate, the most prominent morphology type was recorded after each day of growth for a period of four days.

For the synthetic microfiber work, microfibers provided were collected from a blanket and a portion was treated with radiation to simulate decay over time from UV exposure. MM was made with 5mg of microfibers per 100mL of media. and the microfibers were ground into the media, prior to autoclaving, using a brinkmann homogenizer.

Photomicroscopy was done using a LEICA SYSTEM DM4 B trinocular microscope at 10x magnification, but for the counting of conidia in the microgravity research 40x magnification was also used in cases where the number or presence of conidia was not easily distinguishable.

To simulate microgravity, a clinostat was used which held a number of 16 x 100mm tubes. The clinostat was kept in the same incubator as the controls.

All organisms were incubated at 30°C

CONCLUSIONS

C. albicans and *N. crassa* Results

- No significant changes in morphology were observed between the controls and 0.1nM E2 plates. *C. albicans* grown in YEPD, MM, and Spider remained the same morphology type present within the media control.
- *C. albicans* underwent an inhibition in colony formation when inoculated in microfiber-enriched MM. Colonies in NR microfiber MM were smaller and appeared more jagged and fragmented compared to the more globular colonies in R microfiber MM. Levels of inhibition appeared to be dependent on the concentration of nanofibers in the plate media. In contrast, *N. crassa* underwent no such inhibition and showed no morphological changes when inoculated on microfiber-enriched MM, both for the NR and R microfibers.
- In simulated microgravity, *N. crassa* exhibits a noticeable increase in conidiation. That said, the current dataset is small, and has not been given any thorough statistical analysis. As conidia from the cultures in simulated microgravity tended to be more broken up while those in the control were more often in larger branches together, it is possible this differentiation may be the result of more conidia breaking off rather than increased conidiation.

Future Work

- Subjecting *N. crassa*-inoculated MM plates to simulated microgravity.
- Refining microfiber grinding method so there is consistency in the size of plastic fibers across all plates.
- Subjecting *C. albicans*-inoculated liquid and solid media (YEPD, MM, and Spider) to simulated microgravity.

ACKNOWLEDGEMENTS

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