Comparing Morphological Responses to Stimuli in *Candida albicans* and *Neurospora crassa*

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ABSTRACT

Candida albicans, a yeast found in the human body, and ***Neurospora crassa***, a model filamentous fungi, are two very distinct and distantly related fungi. Although *N. crassa* is well researched, not much is known about *C. albicans*. The objective of our research was to understand the application of *N. crassa* models of morphogenesis when researching *C. albicans*. We exposed *C. albicans* and *N. crassa* to various environmental stimuli under similar conditions. When exposed to osmotic stress hyphal growth was induced in *C. albicans* while *N. crassa* had no significant morphological response to salinity. *C. albicans* was more resistant to osmotic stress than *N. crassa*, which had a significant decrease in growth and survivability as salinity increased. When *C. albicans* was exposed to sudden drops in temperature there was no change in branching pattern, but *N. crassa* has a known cold shock response wherein branching temporarily changes from lateral to apical. When exposed to Estradiol, the amount of filaments in *C. albicans* had a negative correlation to the concentration of Estradiol on the plate. *N. crassa* was also affected by Estradiol qualitatively, often resulting in thinner hypha.

INTRODUCTION

Candida albicans is a fungus that is part of the human microflora. The fungi can be found in moist areas of the human body, such as parts of the skin, mouth, intestines, and vagina. When exposed to certain environmental stimuli (stressors) *C. albicans* has been recorded to undergo a morphological change from a harmless yeast to a filamentous fungus. This is a cause for concern, as filamentous *C. albicans* can use their hyphae to drill into healthy human cells, resulting in an infection known as **candidiasis.**

Neurospora crassa is a filamentous bread mold commonly used as a model organism for fungal and genetics research. Although *N. crassa* is distantly related to *C. albicans*, it is relatively harmless and not part of the human microflora. Thus, our research focuses on investigating the possible applications of *N. crassa* as a model for morphological research on *C. albicans*.

RESEARCH METHODS

Stock cultures of *C. albicans* were stored at 4℃ while stocks of *N. crassa* were stored at -20℃. Plates of *C. albicans* were prepared using the sterile loop-and-streak method. *N. crassa* plates were made by placing a single drop of cells (diluted in sterile water) on the edge of each plate. After inoculation, plates of *C. albicans* were given two days to grow while *N. crassa* plates were left to grow overnight. Minimal (Vogel's) media and Spider media were used for plating. The tested stimuli were salinity (osmotic stress), cold shock, Estradiol (E2), and Fetal Bovine Serum (FBS).

Modified minimal media was made to test **salinity**. A measured amount of NaCl, for each concentration, was mixed into 250 mL flasks of minimal media. The modified media flasks were then autoclaved and made into plates. The tested concentrations were 0.03M, 0.3M, and saturated (~5M).

Minimal plates were used for *N. crassa* **cold shock** while Spider plates were used for *C. albicans*, as spider induced filamentation. Cold shock plates were grown at a higher temperature before being moved to a colder temperature. They were then left to grow in the colder temperature overnight.

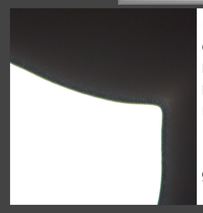
Plates for liquid stimuli solutions, such as **E2** and **FBS**, were prepared by micro-pipetting 80 µl of each solution onto plates and spreading the solution until it was evenly across the surface of the agar. Plates were left to dry before inoculation.

Spider media was used for E2 plates, to observe any possible inhibitory effects E2 may have on *C. albicans* filamentation. The concentrations of E2 tested were 0.1 nM, 1 nM, and 1 µM.

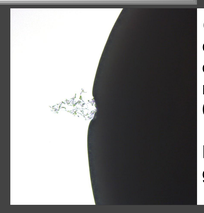
FBS plates were made with plain minimal media. Approximately 1 mL of pure liquid FBS was aliquoted into sterile Ep tubes and inoculated with *C. albicans*.

DATA & RESULTS

OSMOTIC STRESS


C. albicans colony on minimal media with **no NaCl**

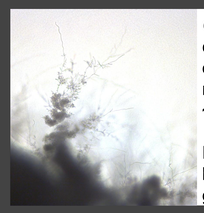
No hyphal growth


C. albicans colony on minimal media with **0.03M NaCl**

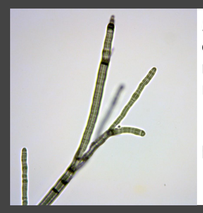
Rare hyphal growth


C. albicans colony on minimal media with **0.3M NaCl**

Occasional hyphal growth


C. albicans colony on minimal media with **~5 M NaCl**

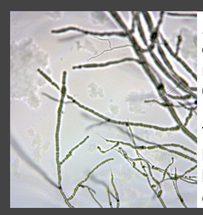
Frequent hyphal growth


N. crassa on minimal media with **no NaCl**

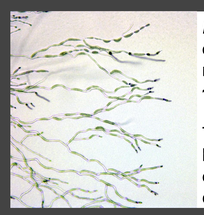
Normal hyphae


N. crassa on minimal media with **0.03M NaCl**

Thin, damaged hypha

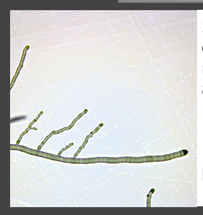

N. crassa on minimal media with **0.3M NaCl**

Thinner hyphae, less gradual curvature

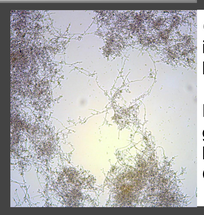

N. crassa on minimal media with **~5M NaCl**

Thinner hyphae, erratic contortions

FETAL BOVINE SERUM


N. crassa on minimal media with **80 µL of FBS**.

Normal branching

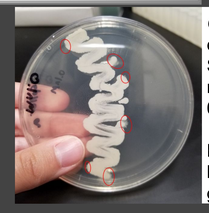

C. albicans in **~1 mL of liquid FBS**.

Hyphal growth in liquid ONLY.

ESTRADIOL (E2)


C. Albicans colony on Spider media with **no E2**

Consistent hyphal growth


C. Albicans colony on Spider media with **0.1 nM E2**

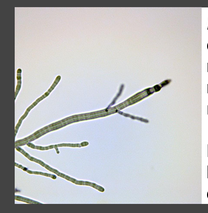
Reduced hyphal growth


C. Albicans colony on Spider media with **1 nM E2**

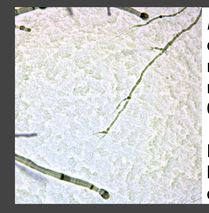
Reduced hyphal growth


C. Albicans colony on Spider media with **1 µM E2**

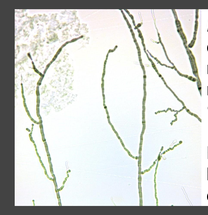
Reduced hyphal growth


N. crassa colony on minimal media with **no E2**

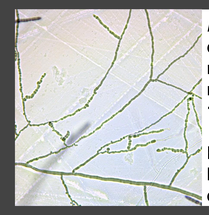
Normal hyphal diameter


N. crassa colony on minimal media with **0.1 nM E2**

Reduced hyphal diameter

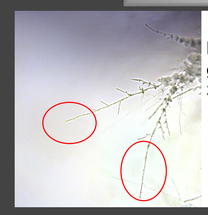

N. crassa colony on minimal media with **1 nM E2**

Reduced hyphal diameter

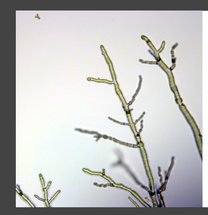

N. crassa colony on minimal media with **1 µM E2**

Reduced hyphal diameter

COLD SHOCK


C. albicans hyphae grown at 30°c


The same hyphae after 2 days of growth at 10°c


N. crassa example normal growth

Lateral Branching


N. crassa example cold shock.

Apical Branching.

CONCLUSIONS

Even when exposed to the stimuli under similar conditions, the morphological responses between C. albicans and *N. crassa* differed greatly. This was to be expected, as both fungi come from very different environments, and thus are exposed to different environmental factors.

- *C. albicans* displayed greater salt tolerance and survivability in salty environments compared to *N. crassa*.
- *N. crassa* displayed a drastic morphological change to cold shock. *C. albicans* had no visible change in morphology.
- *N. crassa* experienced a reduction in hyphal diameter in response to Estradiol (E2) exposure. *C. albicans*, on the other hand, experienced an inhibition of hyphal growth.
- When exposed to Fetal Bovine Serum (FBS), a morphological change from yeast to filamentous was seen in *C. albicans*. *N. crassa* had no visible change in morphology.

FUTURE WORK

- Investigating potential causes of filamentation in *C. albicans* grown in FBS
- Studying conditions in which E2 has different effects on *C. albicans*
- Quantization of the effects of E2 on *C. albicans* under our research conditions
- Potentially grow and test *N. crassa* in liquid media

ACKNOWLEDGEMENTS
This work was supported by award # 1564855 from the National Science Foundation as well as a grant from the Indiana Space Grant Consortium (INSGC).

REFERENCES

1. Smith, D. A., Nicholls, S., Morgan, B. A., Brown, A. J., & Quinn, J. (2004). A conserved stress-activated protein kinase regulates a core stress response in the human pathogen *Candida albicans*. *Molecular biology of the cell*, 15(9), 4179–4190. doi:10.1091/mbc.e04-03-0181