Effect of TNF-α on *C. albicans* Morphological Change in Liquid Media

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INTRODUCTION

C. albicans must undergo a morphological change in order to infiltrate the epithelial cell layer and pose a health threat. This morphological infectious state is known as filamentous due to the elongated protrusion that stems from the base yeast form. To date, the intestinal cues responsible for the change in C. albicans morphology are not clearly defined. Candidiasis is observed in Crohn's disease, where elevated level of inflammatory cytokines such as tumor necrosis alpha (TNF-α) is reported. We hypothesize that TNF-α may represent an intestinal cue responsible for the shift in morphology in C. albicans.

METHODS

- Establishing a test media and positive control:
- We tested our hypothesis in three different liquid media: Minimal (MM), Yeast Extract Peptone Dextrose (YEPD), and Spider media (SM).
- We used Fetal Bovine Serum (FBS) as a postitive control to induce filimentous growth (cite the paper).
- Tubes were incubated at 30°C and growth was evaluated after 2 days under a
- light microscope, specimen scored on whether or not they presented filamentation.
- Evaluating role of TNF-α: 0.1nM TNF-αThese were allowed to grow for 2 days at 30°C and then evaluated and scored for filamentous growth under a light microscope.
- Inoculation: All sample of wild type *C. albicans* were obtained from slants. This was done utilizing a wire loop tool sterilized by use of a bunsen burner.

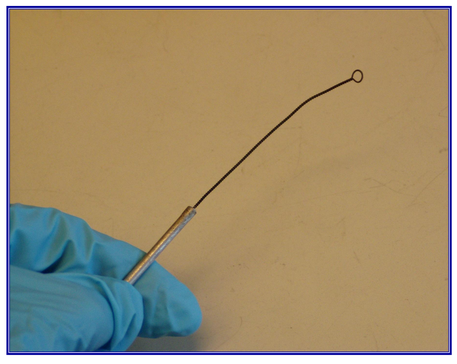

Figure 1

Figure 1: A typical wire or inoculation loop used for this research, image courtesy of wikimedia.org

RESULTS

Figure 2

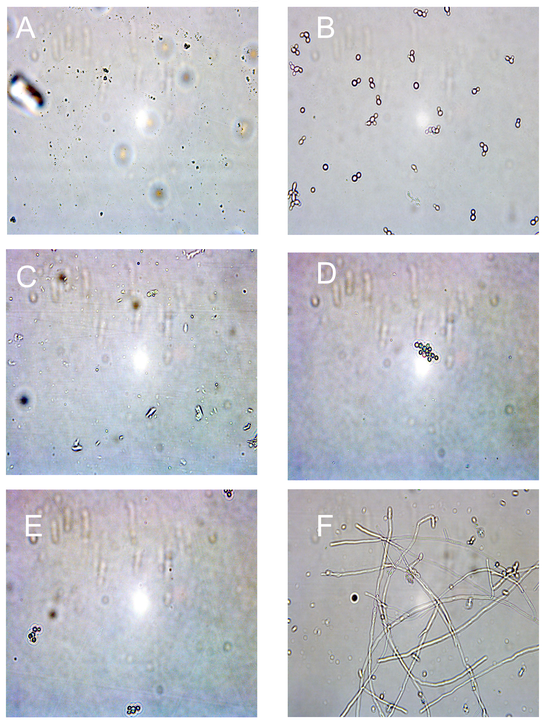

Figure 2: Testing filamentous growth in media
In the top two panels we tested minimal media without (A) and with FBS (B).The middle two panels show YEPD media without (C) and with FBS (D). Finally the bottom pannels show SPIDER media without (E) and with FBS (F)..

Firstly when establishing the conditions for the TNF-α study we found that FBS induced filamentous growth 66% of the time in SM, 16% of the time in YEPD and none in MM (n = 18 plates for each condition). While both YEPD and SM saw significant impact on filamentous growth with FBS, SM showed greater significance so this condition was used in the TNF-α study. In a preliminary study, we found that TNF-α on its own did not cause filamentation (n = 15). Yet, TNF-α totally inhibited FBS-induced filamentous growth, while FBS induced 20% filamentous growth in our control (n=15).

Figure 3

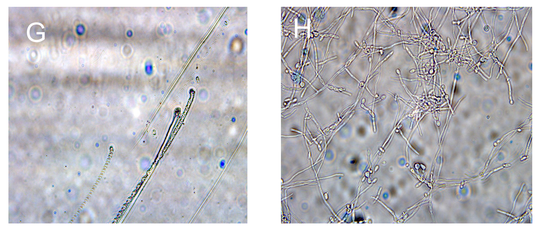

Figure 3: Testing 0.1nM of TNF-α without (G) and with (H) FBS.

CONCLUSIONS

we found that FBS- induced filamentous growth is dependent on the type of liquid media used. In addition, our preliminary experiments suggest that TNF-α prevent filamentous growth and may represent a protective mechanism during candidiasis observed during Crohn's disease. However, there is much to be said for engaging in repeating this experiment even more, and there are plans to do so. Given the discrepancy in our results of filamentous yeast in SM and FBS that is hard to explain, it may just require more testing in the TNF-α. Another possibility is that reexamining the protocol could tease out any possible failures that resulted in skewed data. Additionally, exploring other physiological responses would be beneficial as many responses are made in response to any disease. In particular starting a study featuring estrogen is intriguing given how it has been suggested as being a factor indicating a fatal outcome to sepsis.

References:

1. Mackenzie, D. W. R. (1962). Serum tube identification of Candida albicans. Journal of Clinical Pathology, 15(6), 563–565. doi: 10.1136/jcp.15.6.563