Effect of Media and Estrogen on Morphological Change in Candida albicans

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**INTRODUCTION**

*Candida albicans* (C. albicans), an opportunistic pathogen, lives symbiotically within the intestine of its human host (1). Temperature and chemical factors have been shown to induce a morphological change in *C. albicans* from yeast to filamentous form turning *C. albicans* pathogenic. In this study, we investigated the intestinal cues that might be responsible for the change. We found that different solid media impact the morphological phenotype so we focused on characterizing these before further testing (4). We tested Estradiol (E2) because of its known linkage to sepsis and higher levels during infections (2). Experiments were conducted to compare solid agar plates of YEPD, Minimal Media (MM), and Spider Media (SP) for *C. albicans* growth to choose the best one for further testing with E2 and other factors that could be prone to causing morphological changes (3).

**Material and Methods**

**Models**

*Candida albicans* (C. albicans) was used for testing. Stocks were maintained at 4°C. Parent cultures were renewed every two months.

**Experimental Design**

*C. albicans* was inoculated via streak method on different solid media plates (YEPD, Minimal Media, Spider). Triplicates were made of each media and incubated at 30°C. The effect of 0.1 nM E2 on *C. albicans* morphology was also tested. 80 μL of E2 was spread onto the solid media plates and inoculated with *C. albicans* via streak method. Controls (No E2) for each condition were also made. Experiments were run for approximately 4-5 days with an average of 3 microscope observations per run.

The three observed morphologies were labeled A (smooth-edged), B (tree-like filaments), and C (grass-like filaments). The most prominent morphology type for each plate was recorded during microscope analysis.

**Microscopy**

Morphological changes were visualized using bright-field microscopy. Images were captured using a Leica DM4B microscope alongside Leica Acquire software. *C. albicans* morphology images were captured using both 40x and 5x objective. Images were also taken using a Leica dissecting scope equipped with a digital camera at a brightness of 42%, 3.6x gain, 0.80 gamma, 100% saturation, and a picture magnification of 20x.

**RESULTS**

Figure 1: Effect of different media on *C. albicans* growth and morphology

*C. albicans* was inoculated on triplicates of Minimal Media (MM), Spider (SP), and YEPD media agar plates and incubated at 30°C for 4-5 days, at which point microscope pictures were taken. No growth outside the colony was observed on MM plates, just smooth borders, which was named Phenotype A. On SP plates, *C. albicans* grew in a tree/forest-like shape outside the boundaries of the streaked colony, naming this Phenotype B. YEPD plates resulted in straight and grassy/hairy filamentation outwards from the streak, making it Phenotype C. Out of 6 experiments of 14 MM plates, the expressed phenotype was 86% A and 14% inconclusive of the time. 8 experiments of 17 SP plates showed 100% of phenotype B. 6 experiments of 14 YEPD plates presented phenotype C 92% of the time and 8% inconclusive.

Figure 2: Effect of estrogen on *C. albicans* growth and morphology

These pictures were all taken at 5x magnification. For E2 trials, 2 experiments, 6 MM plates showed 50% phenotype A and 50% inconclusive. 4 experiments, 10 SP plates had phenotype B 100%. YEPD 2 experiments, 2 plates had phenotype C at 100%.

**CONCLUSIONS**

A total of 8 triplicates with 45 plates were run. Minimal media plates were shown to exhibit Phenotype A 86% of the time. Spider plates showed Phenotype B 100%. YEWP plates yielded Phenotype C 92% of the time. For the E2 trials 2 triplicates, 6 MM plates were 50% phenotype A and 50% inconclusive. 4 triplicates, 10 SP plates had phenotype B 100% of the time. As for YEPD, 2 triplicates with 2 plates displayed phenotype C 100% of the time. With the preliminary E2 experiments, the previously characterized phenotypes appear to be consistent with the corresponding media.

**Future Plans**

Experimental conditions for the media controls were established for future testing. Future experiments include whether E2 and other environmental cues, such as inflammatory cytokines, have inhibitory or induction effects on the growth of *C. albicans*.

**References**


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**Table 1: Effect of Estrogen on Morphological Change in Candida albicans**

<table>
<thead>
<tr>
<th>Without E2</th>
<th>With E2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><strong>Spd</strong></td>
<td><strong>Mm</strong></td>
</tr>
<tr>
<td><strong># Plates</strong></td>
<td>45</td>
</tr>
<tr>
<td><strong># Experiments</strong></td>
<td>8</td>
</tr>
<tr>
<td><strong>% A</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>% B</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>% C</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>% Incon.</strong></td>
<td>0</td>
</tr>
</tbody>
</table>