Comparison of Matrix Components and Drug Susceptibility between Candida albicans and non albicans Candida Species

Keely R. Redhage1,2, Heather Taff2, Hiram Sanchez2, David Andes2

Department of Microbiology, Oklahoma State University-Stillwater1
Department of Medical Microbiology and Immunology, University of Wisconsin-Madison2

Abstract
Candida albicans has the capability to form biofilms on medical devices which leads to disease that is costly to treat with high mortality rates. However, infections caused by other Candida species such as C. glabrata, C. parapsilosis, and C. tropicalis have been increasing in frequency. When these pathogens attach to the surface of a medical device, they create a biofilm that is highly resistant to antifungal drugs. Much of this drug resistance is due to the matrix, which essentially creates a barrier preventing drugs from reaching the biofilm producing cells. Proteins and carbohydrates such as, β-1,3-glucan, β-1,6-glucan, and mannans are present in the matrix in high quantities. These components potentially contribute to antifungal resistance of the biofilm and the importance of carbohydrates had been demonstrated in Candida species. This study focuses on matrix components of non-albicans species in comparison to the highly studied C. albicans. My hypothesis is that matrix mannans of non albicans species are similar to those of Candida albicans and are key to antifungal resistance, just as seen for β-1,3-glucan. To collect sufficient matrix material for analysis, biofilms were grown in roller bottles and matrix was harvested after 48 hours. Matrix components were analyzed with phenol sulfuric assay (for total carbohydrate content), and ELISAs (for mannans, β-1,3 glucan, and β-1,6-glucan). We also investigated the role mannans specifically plays in antifungal resistance of non albicans Candida species. XTT assays were used to quantify biofilms following drug treatment with Fluconazole and a combination of Tunicamycin and Fluconazole. We showed that a decrease in matrix mannans causes an increase in fluconazole susceptibility.

Biofilm Formation

- Yeast adherence to surface
- Initial filamentation
- Mature hyphae, matrix production
- Dispersion of new yeast cells away from biofilm

Extracellular Matrix Composition

<table>
<thead>
<tr>
<th>Critical carbohydrate component (by weight)</th>
<th>55% Proteins/Glycoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% Carbohydrates</td>
<td>15% Lipids</td>
</tr>
<tr>
<td>5% DNA</td>
<td>1% s12-branched 1,3,6-mannan (8%)</td>
</tr>
<tr>
<td>1% β-1,3-glucan (14%)</td>
<td>1% β-1,6-glucan (1%)</td>
</tr>
<tr>
<td>1% β-1,3,6-glucan (16%)</td>
<td>1% β-1,6,3-glucan (1%)</td>
</tr>
</tbody>
</table>

Materials and Methods

Collection of Matrix Material:
An individual colony of selected Candida strain was grown with 30 mL of RPMI + MOPS at 37 °C overnight. All culture was then transferred to 2L bottles in 10 mL increments with 80 mL of RPMI + MOPS. Bottles were placed on a rolling device and incubated at 37 °C for 48 hours with one media change after 24 hours. The biofilm was removed from the bottles via scraping and washing with 0.1% SDS. Harvested biofilm was sonicated for 20 minutes followed by collection of the cell pellet through centrifugation at 4 °C for 20 minutes. Matrix supernatant was collected then lyophilized and dialyzed. Cultures were diluted in RPMI + MOPS to 10⁶ cells/ml then grown in a 6 well plate with media changes at 1 and 24 hours. Biofilms were harvested at 48 hours, gently sonicated, and centrifuged to collect matrix suspended in the supernatant. Matrix samples were used for mannans ELISAs and normalized by relative biomass using crystal violet assay.

Carbohydrate Analysis of Extracellular Matrix:
Total carbohydrate matrix components were measured using a phenol sulfuric assay. Mannan concentration was measured using a mannans ELISA. Assays were also run using collected supernatant from six well plates along with bottle grown matrix.

XTT Analysis of Extracellular Matrix:
XTT was used to quantify biofilm viability after treatment with fluconazole, or a combination of tunicamycin and tunicamycin. Cultures were diluted to 10⁶ cells/mL in 100 µL of RPMI + MOPS and then seeded on a 96 well plate for 6 hours to allow biofilm growth. After 6 hours media was gently removed via pipette and washed with 100 µL of 1x PBS. Biofilms were digested with 90 µL of varying concentrations of tunicamycin (1000-125 µg/mL) and tunicamycin (4.0 µg/mL) followed by addition of 30 µL RPMI + MOPS. Plates were incubated for 24 hours and then washed with 100 µL of 1x PBS. Plates were then read with colorimetric XTT assay by treatment with 90µL XTT (0.75 mg/mL concentration) and 10µM PMS (0.32 mg/mL, concentration) in a dark room. Plates were incubated for 30 minutes, in the dark, at 37 °C and then read at OD 492 using a microtiter plate reader.

Results

Amount of Matrix per sample

- The focus of this study was the extracellular matrix components of the following Candida species:
  - C. glabrata
  - C. parapsilosis
  - C. tropicalis
- Strains were analyzed for the following content:
  - Total Carbohydrates
  - Mannan

Materials and Methods

Comparison of Matrix Components and Drug Susceptibility between Candida albicans and non albicans Candida Species

Results

- Carbohydrates were found in C. glabrata, C. parapsilosis, and C. tropicalis
- Significant amounts of mannans were present in all tested non albicans Candida species
- The non albicans Candida strains of C. glabrata, C. parapsilosis, and C. tropicalis all showed increased susceptibility with the addition of Tunicamycin to Fluconazole
- Addition of Tunicamycin to Fluconazole did not significantly increase the susceptibility for C. parapsilosis

Conclusions/Future Directions

Amount of carbohydrates found in non albicans Candida species is similar to that of Candida albicans
- C. parapsilosis produced higher levels of matrix per cell than C. albicans and other non albicans species (Figure 1)
- C. parapsilosis has less relative mannans and this could explain the inability of tunicamycin to affect drug susceptibility as shown in figure 2
- Future studies would be an analysis of other extracellular matrix components

Acknowledgements

I would like to extend my gratitude to the entire Andes lab for their help this summer, without you this project would not have been possible. I would especially like to thank Heather Taff for her patience and guidance while completing my project. Thank you Dr. Andes for allowing me to work in your lab and my program director, Dr. Roll, for this wonderful opportunity. I am extremely grateful to my family for their love and support in all my academic endeavors, thank you for always believing in my success. Funding provided by NSF

References