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

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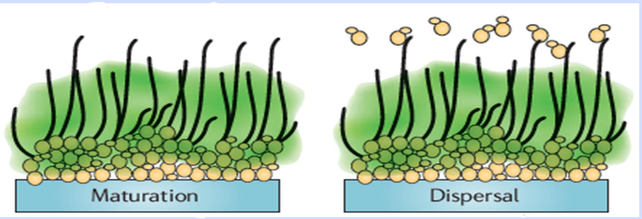
Abstract

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 mannan 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 mannan 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.[1] 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 mannan, β-1,3- glucan, and β-1,6-glucan). We also investigated the role mannan 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 mannan causes an increase in fluconazole susceptibility.

Biofilm Formation

•Yeast adherence to surface

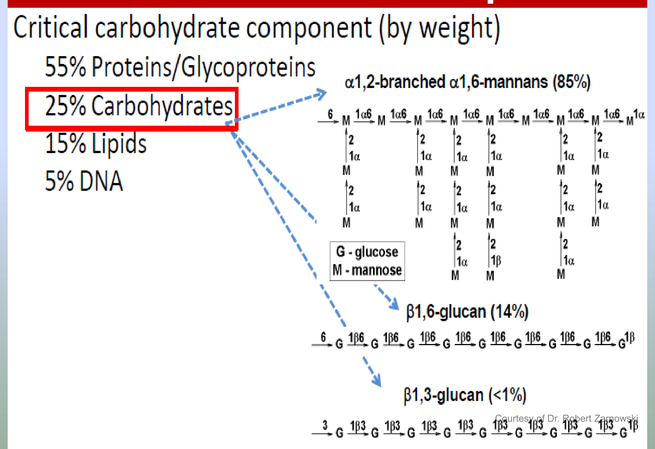
•Initial filamentation



•Mature hyphae; matrix production

• Dispersion of new yeast cells away from biofilm

Extracellular Matrix Composition



Focus

•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

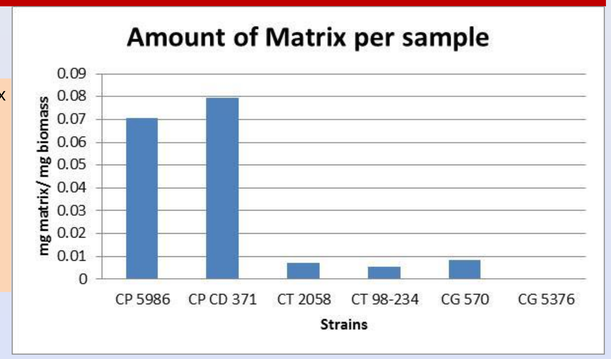


Figure 1:
Amount of matrix and biomass was quantified after growth in roller bottles. High matrix per biomass represents strains with cells that produce high levels of matrix. Low matrix per biomass represents strains with cells that produced low levels of matrix. CG 5376 did not grow in roller bottles therefore no matrix could be collected.

Materials and Methods

Collection of Matrix Material:

An individual colony of selected *Candida* strain was grown with 30 mL of RPMI + MOPS at 37 ºC 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 ºC for 48 hours with one media change after 24 hours. The biofilm was removed from the bottles via scrapping and washing with 0.1% SDS. Harvested biofilm was sonicated for 20 minutes followed by collection of the cell pellet through centrifugation at 4 ºC for 20 minutes. Matrix supernatant was collected then lyophilized and dialyzed.

Cultures were diluted in RPMI + MOPS to 10^6 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 mannan 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 mannan 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 flucanozole, or a combination of fluconazole and tunicamycin. Cultures were diluted to 10^6 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 pipitte and washed with 100 µL of 1x PBS. Biofilms were dosed with 90 µL of varying concentrations of fluconazole (1000-125 µg/mL) and tunicamycin (4-0 µg/mL) followed by addition of 90 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 10µl PMS (0.32 mg/mL concentration) in a dark room. Plates were incubated for 30 minutes, in the dark, at 37 ºC and then read at OD 492 using a microtiter plate reader[2].

Results

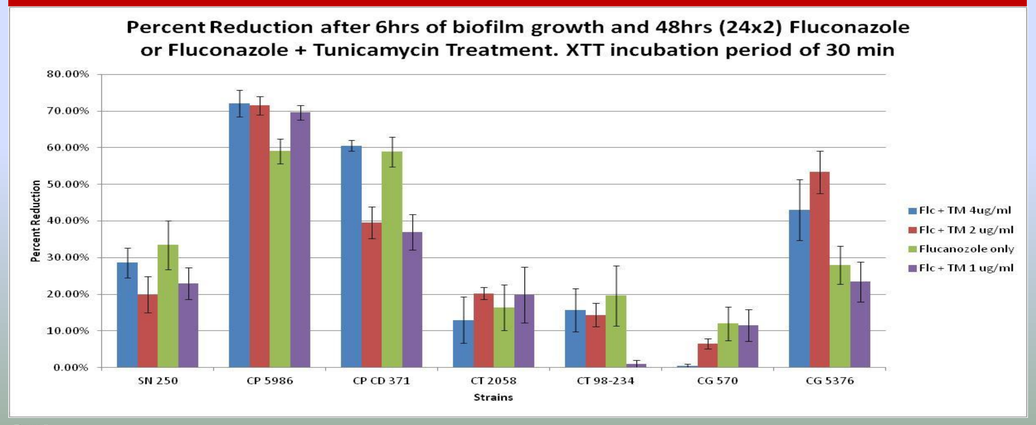


Figure 2:
Biofilms were grown in 96 well polystyrene plate for 6 hours. Media was then removed and exchanged with either fluconazole, tunicamycin or a combination of both. XTT was used to quantify biofilms at 48 hours and read at OD 492. Data shown is 500 ug/ml fluconazole concentration.

Results

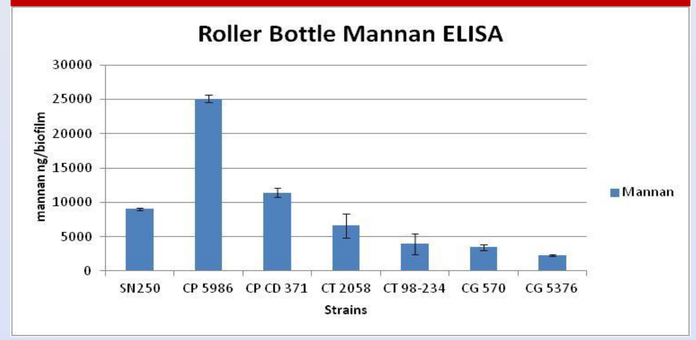


Figure 3:
Biofilm was grown in 2L roller bottles according to methods as described above. Biofilm was harvested and purified matrix was analyzed through an indirect mannan ELISA. Collected data was normalized with respect to mg of matrix for the given sample.

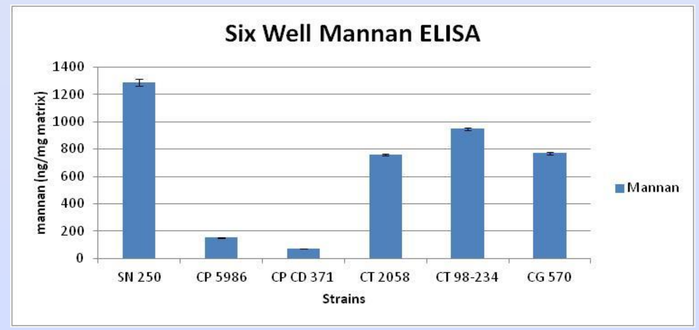


Figure 4:
Biofilm was grown in six well plates according to methods as described above. Biofilm was harvested and matrix was analyzed through an indirect mannan ELISA. Collected data was normalized by relative biomass using crystal violet assay

- Carbohydrates were found in *C. glabrata, C. parapsilosis*, and *C. tropicalis*
- Significant amounts of mannan 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 Flucanazole
- 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 matrix mannan 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

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References

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2. Nett JE, Cain MT, Crawford K, Andes DR. Optimizing a Candida biofilm microtiter plate model for measurement of antifungal susceptibility by tetrazolium salt assay. *J Clin Microbiol*. 2011 Apr;49(4):1426-33.