Characterization of Morphogenesis in a SKN7/MBP1 Double Null Mutant Strain of Candida albicans

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Abstract

Candida albicans is a fungal pathogen that can cause opportunistic infections as well as systemic infections in immune-compromised patients.(2) It has been well established that morphogenesis, the transition from yeast to filamentous growth forms, is essential for *C. albicans* to cause systemic infections.(1) Previous work in our lab has shown that the Mbp1 protein is required for morphogenesis under nitrogen-limiting conditions on solid media. In addition, it has been reported that strains of *C. albicans* that lack the Skn7 protein exhibits reduced morphogenesis in response to serum and the lack of a fermentable carbon source.(3) In this study, morphogenesis was assessed in a double null mutant strain of *C. albicans* that lacks both the Mbp1 and Skn7 proteins. The results of this analysis indicate that compared to the wild type strains, SC5314 and CAI4, there was a significant reduction of morphogenesis in response to limiting nitrogen, pH and a non-fermentable carbon source in the MBP1/SKN7 double-null mutant strains. In addition, morphogenesis in response to fetal bovine serum was highly variable in the double-null mutant strains. These results indicate that the Mbp1 and Skn7 proteins may not function in the same signal transduction pathway, but could possible interact.

Materials and Methods

Morphogenesis in Solid Media

1. Grow overnight broth cultures (5.0ml) of yeast strains in YPD + 25 µg/ml uridine as needed
2. Dilute broth cultures (9.0ml) to 0.5 McFarland Standard
3. Dilute 0.5 McFarland Standard culture to $10^{-2.5}$
4. Inoculate 100µl of diluted broth culture onto solid morphogenesis media (10%FBS, M199pH 7.5, SLAD, and Spider; supplement with 25 µg/ml uridine as needed)
5. Incubate cultures in 30C or 37C Incubator for 5 days
6. Take pictures and then incubate for additional 3 days

Microscopy

1. Olympus Microscopic Camera with Leica software
2. Magnification varied between 7x to 45x depending on colony size
3. Take pictures after 5 days of culture incubation on solid morphogenesis media and again after 8 days

Results

Figure 1: Morphogenesis Results for *Candida albicans* Strains Grown on Morphogenesis Inducing Media

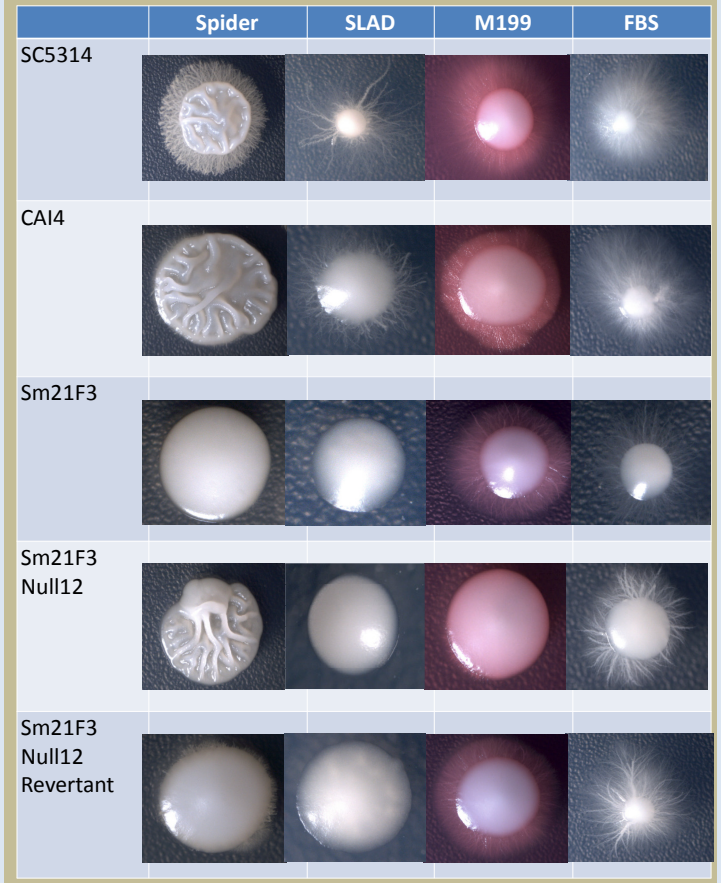

Table 1: Genotypes of *Candida albicans* Strains

Strain	Relevant Genotype
SC5314	Wild Type
CAI4	*Δura3::imm434/ Δura3::imm43*
Sm21F3	*Δura3::imm434/Δura3::imm434 Δskn7::hisG-URA3-hisG/SKN7 Δmbp1::hisG/Δmbp1::hisG*
Sm21F3 Null 12	*Δura3::imm434/Δura3::imm434 Δskn7::hisG-URA3-hisG/Δskn7::hisG Δmbp1::hisG/Δmbp1::hisG*
Sm21F3 Null 12 Revertant	*Δura3::imm434/Δura3::imm434 Δskn7::hisG-URA3-hisG/SKN7 Δmbp1::hisG/Δmbp1::hisG*

Discussion

There is a significant reduction in morphogenesis of the double null strains on all media (M199, SLAD, Spider) except for FBS compared to the wild type strains. Disruption in morphogenesis is displayed on the double null strains specifically on M199 media when contrasted to the heterozygous strain (Sm21F3). The reduction and disruption in morphogenesis demonstrates that the Mbp1 and Skn7 proteins may not function in the same signal transduction pathway regulating morphogenesis, but could possibly interact. The procedure was completed once in 30C incubation and once in 37C incubation. The results from the 37C incubation displayed similar findings differing by larger radius of colonies and marginally accelerated morphogenesis. There is slight variability of morphogenesis amongst colonies, thus replication is necessary.

References

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