Quantifying the effects of multicellular mutations TUS2 and AIM44 on size and growth rate in *S. cerevisiae*

By Emily Sroga and Erik Wanberg

Introduction

Following the breakthrough of selection-induced multicellular yeast in 2011, new questions rapidly emerged within the field of microevolution.[1] Using a settling selection method, where yeast cultures are allowed to quickly settle, and only the bottom colonies are selected for further plating, researchers were able to induce multicellularity in otherwise unicellular yeast--creating a potential look into the origins of multicellular life.[1] Several multicellular mutations were identified and sequenced, leading to further opportunities to better understand the mechanisms and applications of multicellular yeast formation. Two of these mutations, named TUS2 and AIM44, were the focus of our project. To better understand how these mutations lead to multicellularity, as well as determine the differences between them and the other mutations, we examined TUS2 and AIM44, along with the wild type strain Y55, through a series of plating and subsequent colony analysis. Through our procedure, we gained insight into the differing sizes and growth rates of the mutated strains.

Methods

Cell cultures

Cell cultures were made from ancestral strains of *S. cerevisiae* with the wild-type genotype (Y55), TUS2 mutation, and AIM44 mutation. Three clones were propagated from each strain in YPD media.[2] Samples of each clone were taken and grown in conditions of both selection and non-selection. Three replicates were made for each clone in each condition. Passes were made daily for a total of ten days.

The selection procedure involved the cell culture sitting for several minutes to allow denser cells to sink to the bottom before passing a sample from the lower portion of the tube onto the next generation.[1] This process selects for multicellular colonies over unicellular.

Plates

Plates were made from samples of the cell cultures every other day of passing to check for contamination and observe physical differences in colonies. Plating serves as a primary way to recognize the evolution of multicellularity, as the variations between multicellular and unicellular *S. cerevisiae* are easily visible.

Size distribution

A coulter counter machine was used with samples from the final day of the experiment to visualize the distribution of cells by size.[3] The cell density at various cell diameters was measured for each sample. This serves as another indicator of multicellularity, as multicellular *S. cerevisiae* will exhibit a significantly higher diameter than unicellular *S. cerevisiae*. This machine was also utilized later on in the experiment to observe size differences between select AIM44 cell cultures.

Growth rate

A tecan instrument was used to measure the OD600 over a period of 22 hours of each ancestral clone.[4] These measurements were used to visualize the growth of each strain and identify any differences.

Results

Figure 1 depicts the size distributions, as measured by the coulter counter, of samples taken from each strain in both settling and non-settling conditions. Most notably, AIM44 exhibited a unique bimodal distribution, with peaks at around 3.5 and 5.4 micrometers. AIM44 non-settling samples also deviated from the TUS2 and Y55 strains in size. AIM44 NS peaked at around 4.5 micrometers, which was significantly less than that observed in TUS2 and Y55. This peak also varied significantly from the previously mentioned AIM44 S sizes, as calculated by their respective spans. The span difference between these selection treatments was found to be significant ($p<0.0001$).

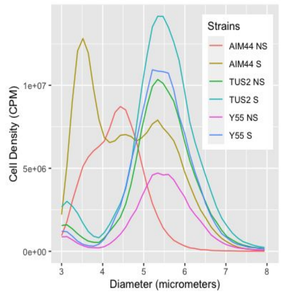

Figure 1. Cell density grouped by diameter for AIM44, TUS2, and Y55 after both settling (S) and non-settling (NS) selection. A coulter counter was used to measure the cell density in counts per milliliter (CPM) of culture samples at specific diameters after 10 days of growth. Five replicate readings were conducted for each sample and an average CPM was found for each strain and treatment. Outcomes of non-settling and settling selection within each strain were compared for their difference in variance, as calculated by their span. The difference in span between selections of AIM44, TUS2, and Y55 were found to be significant ($p<0.05$).

To further analyze the variance seen in AIM44 samples, we selected and propagated both small and large colonies and ran them again through the coulter counter. Figure 2 shows the results of this run, along with a photograph of the small and large colonies. The differences were extremely apparent even in the petri dish, so our coulter counter results were expected.

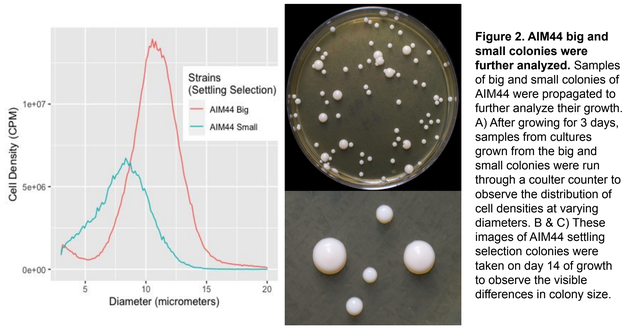

Figure 2. AIM44 big and small colonies were further analyzed. Samples of big and small colonies of AIM44 were propagated to further analyze their growth. A) After growing for 3 days, samples from cultures grown from the big and small colonies were run through a coulter counter to observe the distribution of cell densities at varying diameters. B & C) These images of AIM44 settling selection colonies were taken on day 14 of growth to observe the visible differences in colony size.

Figure 3 displays the growth curves produced by the tecan instrument. As seen on the graph, AIM44 samples exhibited consistently lower rates of growth than the other two strains. A significant difference between OD600 values from the varying strains was found ($p<0.05$).

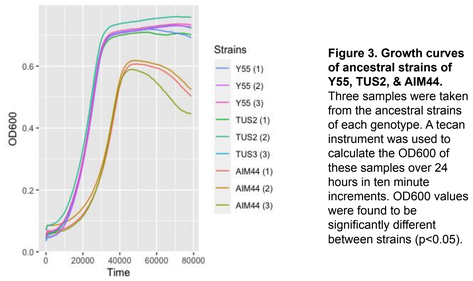

Figure 3. Growth curves of ancestral strains of Y55, TUS2, & AIM44. Three samples were taken from the ancestral strains of each genotype. A tecan instrument was used to calculate the OD600 of these samples over 24 hours in ten minute increments. OD600 values were found to be significantly different between strains ($p<0.05$).

References

[1] Ratcliff, W. C., Denison, R. F., Borello, M., & Travisano, M. (2012, January 31). Experimental evolution of multicellularity. PNAS, 109(5), 1595-1600. https://doi.org/10.1073/pnas.1115323109

[2] Treco, D. & Lundblad, V. (1993, July). Preparation of Yeast Media. Current Protocols. Retrieved from https://doi.org/10.1002/0471142727.mb1301s23

[3] Zhang, W., Hu, Y., Choi, G., Liang, S., Liu, M., & Guan, W. (2019, October 1). Microfluidic multiple cross-correlated Coulter counter for improved particle size analysis. Sensor and Actuators B: Chemical, 296. https://doi.org/10.1016/j.snb.2019.05.092

[4] Bukhman, Y.V., DiPiazza, N.W., Piotrowski, J. et al. (2015). Modeling Microbial Growth Curves with GCAT. Bioenerg. Res. 8, 1022–1030. https://doi.org/10.1007/s12155-015-9584-3

[5] Harai, Y., et al. (2018, March 19). Spontaneous Changes in Ploidy Are Common in Yeast. Current Biology, 28(6), 825-835. https://doi.org/10.1016/j.cub.2018.01.062

Conclusions

The size discrepancy present in both AIM44 selections may be due to haploid conversion caused by the yeast's selecting environment. Yeast is generally diploid or polyploid, and size generally increases with a greater number of chromosomes. While haploid yeast cells are less common due to their stability, they can still be found in nature. Haploid cells are usually smaller, which may explain the decreased AIM44 S small colony sizes.

The low growth rates of AIM44 samples do not necessarily explain the different colony sizes seen in AIM44 selection samples. In the case of AIM44 S for example, small and large colonies existed in the same growth period, implying a genetic difference likely exists outside of their rate of growth. In addition, the growth rate was measured before any selection was conducted. No other significant results were observed from our growth rates.

Future Directions

To further determine if the physical differences in AIM44 cell size are genetically based, we may consider sequencing the small and large colonies. It would also be worth confirming the ploidy level of the small and large colonies using flow cytometry. If the cause of the phenotypic differences is genetic, this would help to establish if the changes occur at a DNA or chromosomal level.

Due to the time constraints of our experiment, only one of our samples exhibited multicellular growth. It may benefit us to run the same experiment for a longer period of time with the hopes of evolving more multicellular yeast. We could then see if the size differences between strains of unicellular colonies are also experienced at the multicellular level.

In addition, it would be interesting to observe the physical characteristics of the other multicellular mutants that were previously identified. This could introduce new variations in size or growth that weren't experienced in the strains we focused on.