Comparison of the Fitness of W303 Yeast and BC187 Yeast When Under ER Stress

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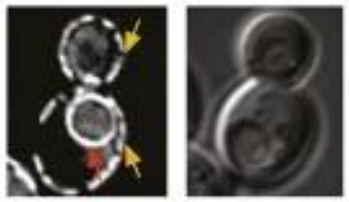
Abstract

"ER stress" is a condition of the endoplasmic reticulum (ER), an organelle compartment present in the eukaryotic cell, that is caused by either an accumulation of unfolded proteins in the ER or an increase in demand in the cell for the generation of secreted proteins such as insulin. In this study, I compared the abilities of two strains of the yeast *Saccharomyces cerevisiae* to respond to this stressed condition. The first of these was the strain typically used in research labs and the other was a strain extracted from a winery. In order to asses the ability of these strains to handle ER stress, I compared their growth rates using various methods with and without the ER stress condition. I found that the wine strain of yeast grew faster than the lab strain in normal and stressed conditions, suggesting that the wine-making process and environmental conditions of the wine yeast strain may have benefitted its general fitness. I also allowed the yeast cells to age over time to analyze the affect of aging on the cell's ability to handle the stress and found that the aged wine strain cells grew less colonies than the lab strain, which suggests that the strain loses its tolerance over time or that the tolerance of the lab strain increases.

Introduction/Background Information

Budding Yeast Cell with ER



Yeast

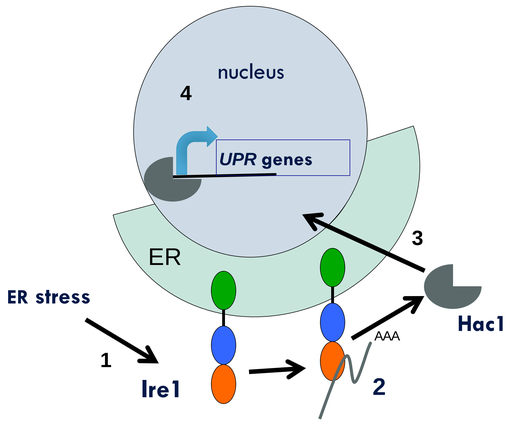
Yeast are single-celled organisms that reproduce through budding, as pictured above. The strains of the *S. cerevisiae* yeast that were used in this study included W303, the lab strain, and BC187, the wine strain.

ER (endoplasmic reticulum)

The endoplasmic reticulum is a cell organelle present in eukaryotic cells that is connected to the reticular network. It is a site of protein synthesis, folding, and modification for secretory proteins and a site of lipid synthesis. Yeast cells contain cortical ER (cER) near the plasma membrane, pointed yellow above, and perinuclear ER (pER) around the nucleus, pointed red above.

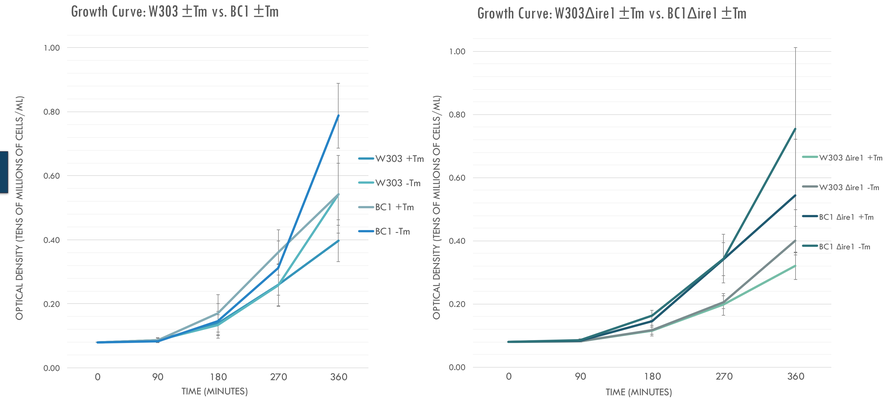
UPR (unfolded protein response)

The Unfolded Protein Response is a pathway activated by ER stress, as shown below. In research labs, ER stress can be experimentally induced through the use of Tunicamycin (Tm), a chemical that inhibits glycosylation of proteins in the ER. After ER stress is induced, it activates the protein kinase Ire1, which is located on the ER membrane. This is the start of a signal transduction pathway, the UPR, that ends with the transcription and translation of genes that work to remove the stress.



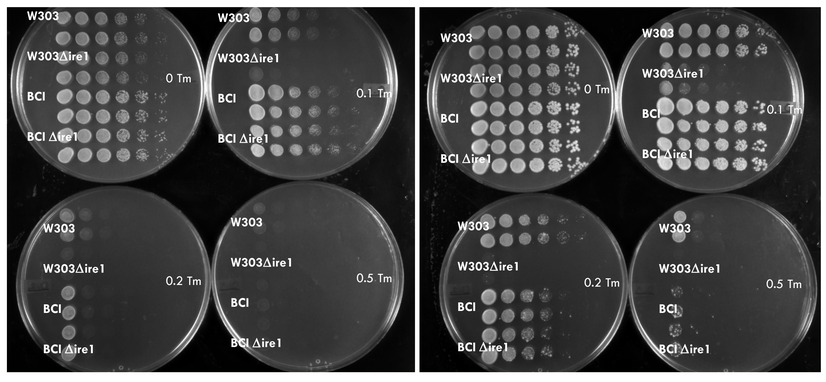
Results

1) I grew both strains of yeast with and without Tm over a period of 360 minutes in order to measure the difference in growth when under ER stress. I also experimented with growing them with their ire1 coding gene knocked out to see the significance of the ire1 protein kinase in a cell's ability to handle ER stress. The four strains were inoculated and grown overnight. After diluting each strain to the same cell density, I used the spectrophotometer to measure the OD, or optical density, of each strain in 90 minute periods. OD is read in tens of millions of cells.



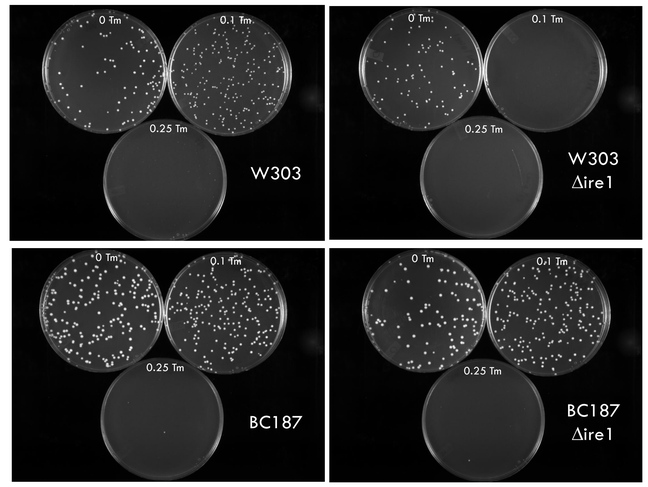
The collected data shows that the BC187 strains, retrieved from the winery, grew at much faster rates than the lab strains in all conditions (under and out of stress, with and without ire1). This suggests that the environment of the winery may have affected the yeast strain is a way that allows is to better reproduce. You can also observe that the lab strain was much more affected by the knockout of ire1 than was the wine strain when both with and without tunicamycin. This suggests that the strain from the winery may have developed other mechanisms for controlling ER stress.

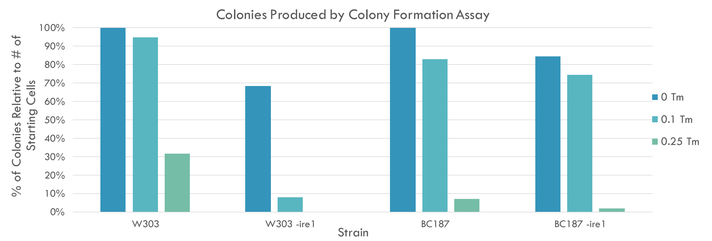
2) Another method that I used to compare the growth and sensitivity of these four strains under ER stress was through a dilution series growth assay. In 4 plates with increasing amounts of Tm concentration (0, 0.1, 0.2, 0.5 μg/mL) I grew increasingly diluted amounts of each strain to better see how they were affected by the stressed conditions and how they fared without the *IRE1* gene.



Observation made from looking at the results of this experiment reaffirmed those that were made from the graphs of the growth curve. The wine strains seem to be growing better than the lab strains. The affect of the knockout of the ire1 gene strain is greatly shown in the lab strain, as it grew at a much slower rate when ER stress was introduced, whereas the BC187 strains show almost no difference between the wild type and mutant. The growth of all four strains gets worse as the Tm concentration, or ER stress intensity, gets higher.

3) The third part of the comparison of these strains was done to observe how age of the cells affects their ability to handle the ER stress. After inoculation, the cells of each of the four strains (W303, W303 Δire1, BC187, BC187 Δire1)were allowed to grow and age for five days before they were retrieved for the experiment. I then diluted them to a concentration of approximately one cell per milliliter and spread them on plates of increasing Tm concentrations (0, 0.1, 0.25 μg/mL). The number of colonies successfully grown on the plates show were then counted and used as data to show the fitness of the respective strains in their respective conditions.





Through observations on the older yeast cells, we see that the wild type W303 cells survive better than the BC187 cells do. A larger percentage of the W303 cells thrive in the ER stressed environments. This is not true for the older ire1 knockout strains. In these, the wine strain grows more colonies than the lab strain, showing better fitness as it does before the aging process. This suggests that the BC187 cells somehow lose their ability to better withstand stressed conditions over time or that the tolerance of the W303 cells increases to high than the wine strain. We can also suggest that, due to not having as significant a role for the ire1 gene as the W303 cells, the BC187 cells still grow better than them without it.

Discussion and Conclusions

Overall, the major observations and final results of this study were that the wine yeast strain, BC187, grows at a much faster rate than the typical lab strain, W303, and shows better fitness in environments that both contain ER stress and those that do not, suggesting a positive affect of the environment of the wine strains on their fitness. We also see that with increasing levels of Tm and, therefore, ER stress intensity, the fitness of both of these strains decreases as expected, but the removal of the ire1 gene, a key factor of the UPR, has a large impact on the fitness of the W303 cells and almost no impact on the BC187 cells. This suggests that the wine strain may have other mechanisms to work against ER stress. As the cells get older, however, the increased fitness of the wine strain decreases and the W303 cells survive better than the BC187 cells. In the older cells with the ire1 gene knocked out, we see that the BC187 cells once again thrive better than the lab strain. This suggests that the BC187 cells may lose their ability to endure high stress levels over time or W303 cells may increase their tolerance of the stress as they get older. However, the wine strain still has more colonies than the lab strain when ire1 is knocked out, possibly due to being less reliant on the protein kinase.

References

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