



## Abstract

Understanding the oxidative stress response of eukaryotic cells in relationship to reactive oxygen species (ROS) can open up many doors to understanding, and thus possibly curing, diseases caused by exposure to ROS such as cancer and neurodegenerative disorders. Previous studies have indicated hexose transport genes (HXTs) may significantly influence stress response of the eukaryotic organism *Saccharomyces cerevisiae*.

This study follows *HXT2* mutants compared to normal strains of *S. cerevisiae* with the hypothesis that *HXT2* mutants will show a significant decrease in growth when exposed to oxidative stress; varying results that show a possible correlation between *HXT2* status and oxidative stress sensitivity, with further research needed for definitive support.

## Introduction

- The HXT superfamily is responsible for hexose transportation in *Saccharomyces cerevisiae*. Here, I have focused on *HXT2* and its effect on the cellular response to oxidative stress. The *HXT2* gene encodes a high affinity glucose transporter of the major facilitator superfamily and its expression is induced by low levels of glucose and repressed by high levels of glucose which may play a key role in stress response.
- Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and their removal by antioxidant defenses. ROS are reactive molecules and free radicals derived from molecular oxygen.
- An ROS at low exposure and dosage such as hydrogen peroxide, which is the stressor used in this study, will promote stress response and cell proliferation in certain types of cells.
- I hypothesized that removing the *HXT2* gene in *S. cerevisiae* will cause increased cell death during the stress response in comparison to cells with intact *HXT2* (wild type BY4741).
- If *HXT2* is not present, the cells will likely struggle to successfully transport glucose while under stress and in turn, this will increase cell death.

## Methods

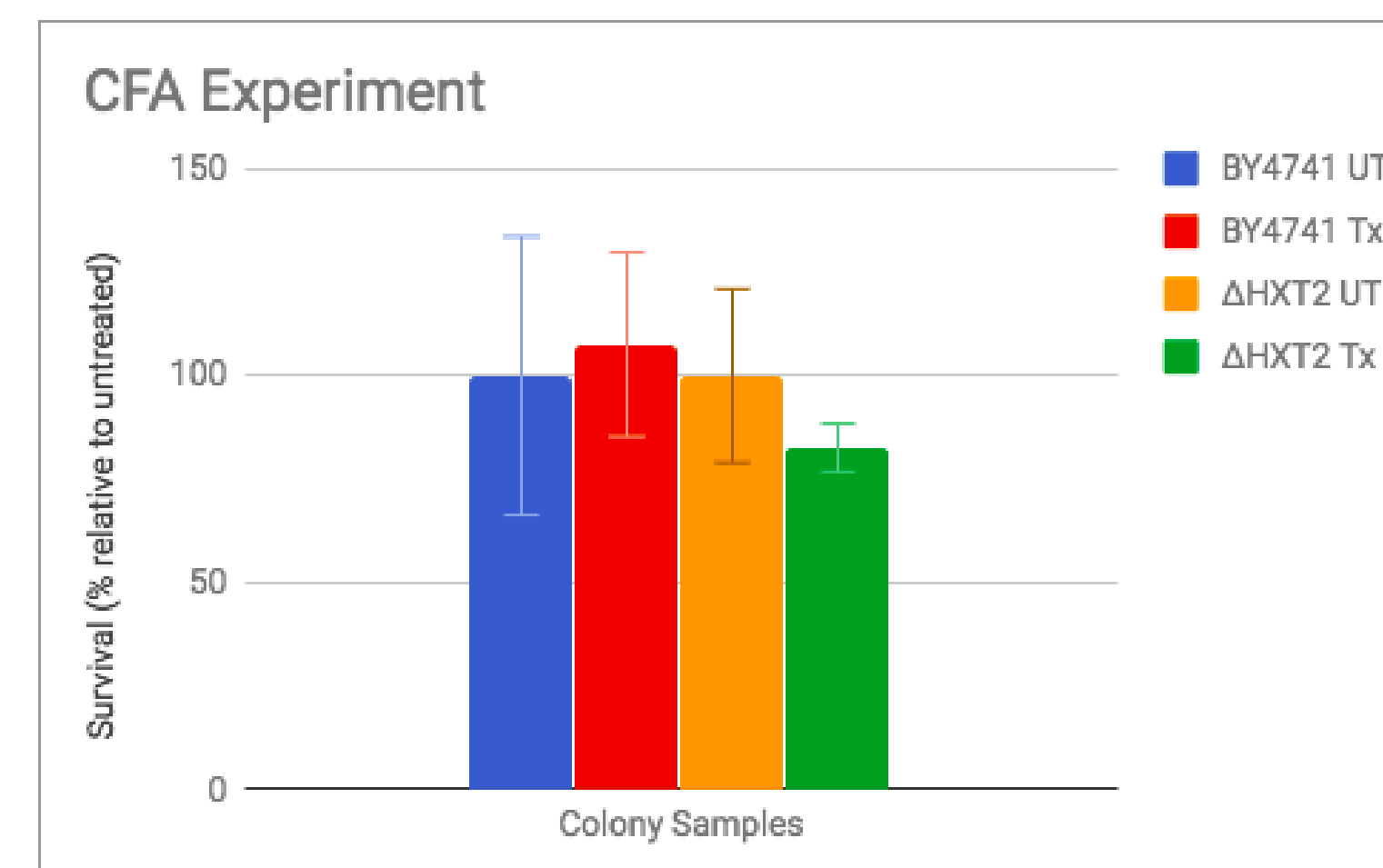
- The experiment follows a lab protocol for a colony-based *S. cerevisiae* sensitivity assay. The strain with deleted *HXT2* was previously acquired and will be referred to as *hxt2-Δ* for the remainder of this study; the wild-type BY4741 (parent to *hxt2-Δ*) is commercially available.
- I prepared plates with appropriate media (yeast extract, peptone dextrose, or YPD) and grew an overnight culture from one colony of each strain.
- The next day, I utilized a spectrometer to measure the absorbance of visible light at 600 nm to determine the concentration of the yeast cells in culture. The ideal optical density for this experiment is between 3 and 10, indicating the culture is in stationary growth phase.
- Based on this measurement, I then diluted each overnight culture in YPD to an optical density equal to 3.
- Hydrogen peroxide was used to induce oxidative stress in the cells at a final concentration of 20 mM. To test our hypothesis that *hxt2-Δ* strain will show significantly higher cell death in stress response than the BY4741 strain, I labeled four tubes: BY4741 untreated (control tube), BY4741 treated, *hxt2-Δ* untreated (control tube) and *hxt2-Δ* treated.
- The treated tubes contained the appropriate amount of culture, YPD, and hydrogen peroxide while the untreated tubes contained appropriate amount of culture and YPD; all four tubes were then allowed to grow in an incubator at 30° C, 220 RPM for 1 hour.
- After this exposure, I completed a total of 6 serial dilutions. Using the appropriate 10<sup>-6</sup> diluted sample, I plated four 100-μL samples of both untreated and treated samples of BY4741 as well as *hxt2-Δ* for a total of 16 plates. These plates were incubated for approximately 48 hours and then the colonies were counted.

## Results

Results supporting the hypothesis would have shown survival from greatest to least in this order: BY4741 Untreated (UT), BY4741 Treated (Tx), *hxt2-Δ* Untreated (UT), and *hxt2-Δ* Treated.

The observed results showed BY 4741 Tx to have a slightly increased number of colony relative to BY 4741 UT. There is a small but inconclusive decrease in survival of the *hxt2-Δ* strain relative BY 4741 Tx, due to the variability associated with this experiment.

It is important to note that this graph represents technical replicates (n=4) of a single experiment; in order to further prove the hypothesis, the experiment would have to be completed on multiple days, perhaps including other HXT deletion strains (i.e. *hxt4-Δ*) as candidate oxidative stress-responsive genes.



CFA Experiment		
	Colony Samples	StDev
BY4741 UT	100	33.614
BY4741 Tx	107.503	22.243
ΔHXT2 UT	100	20.965
ΔHXT2 Tx	82.637	5.789

## Discussion

The results were inclusive. Further studies are required to determine if the hypothesis is supported. The results do, however, support that cells without the *HXT2* gene grow less efficiently under oxidative stress than cells with the *HXT2* gene, which is represented by BY4741. An explanation for BY4741 Untreated having the less growth compared to BY4741 Treated could be that there was a contamination of the master plate or insufficient nutrients and conditions for growth on the agar plates, since this result was observed in other (replicate) experiments.

It is possible that *HXT2* is less beneficial to the cell under favored conditions but is valuable under poor conditions where the cell is under oxidative stress by assisting in the transportation of glucose in order to balance the free radicals present. It is also possible *HXT2* works alongside other HXT family genes, such as *HXT4*, in order to overcome oxidative stress. In addition, eukaryotic organisms exhibit many different processes and there is likely a "back up" process which may become active during oxidative stress when *HXT2* is not present, this alternative process may explain the difference in growth.

## Reference:

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