Phenotypic testing of *YIL055C* deleted *Saccharomyces cerevisiae* strains

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Introduction:

Saccharomyces cerevisiae, or bakers' yeast, has had its genome sequenced for decades, but many of the genes still have no known or understood function. The Yeast Genome Project has sought to connect researchers from across the world toward the goal of creating a complete gene map and database for the genome of Baker's Yeast. To do this genes are labeled with Gene Ontology (GO) terms. These Gene Ontology terms allow those looking at the database to quickly understand the cellular component (location), the Molecular function, and what Biological process the gene and its protein derivatives are a part of. The current Database lists the GO terms for YIL055C as Unknown for both biological process and for Molecular function and suggests a Mitochondrial cellular component. To identify gene functions a *YIL055C* deletion mutant was constructed alongside a Green Fluorescent Protein (GFP) labeled strain, and multiple phenotypic tests were performed to acquire data. By understanding and identifying *Saccharomyces cerevisiae* genes the scientific community will gain more knowledge for identifying genes of unknown function in other species as well.

Background:

We previously, used bioinformatics to help understand the nature of the YIL055C gene. In sum, bioinformatic investigations revealed that YIL055C likely produces a protein product as it has several orthologs surrounded by similar genes in other organisms. It was reported on SGD that YIL055C-p localized to the mitochondrion in a large-scale study, but no individual supporting evidence was provided. In addition, bioinformatic investigation suggested localization to either the mitochondria the nucleus, prompting further investigation. Phenotypic and interaction investigation suggests YIL055C may be involved in vacuolar morphology and cellular response to an oxidative stress environment.

Oxidative Stress Growth Test

The growth rates of Wild type and YIL055C deletion mutants in an oxidative stress environment suggest that the YIL055C gene has an affect on the yeast organism's ability to survive in environments of high oxidative stress as seen in Figure 2.

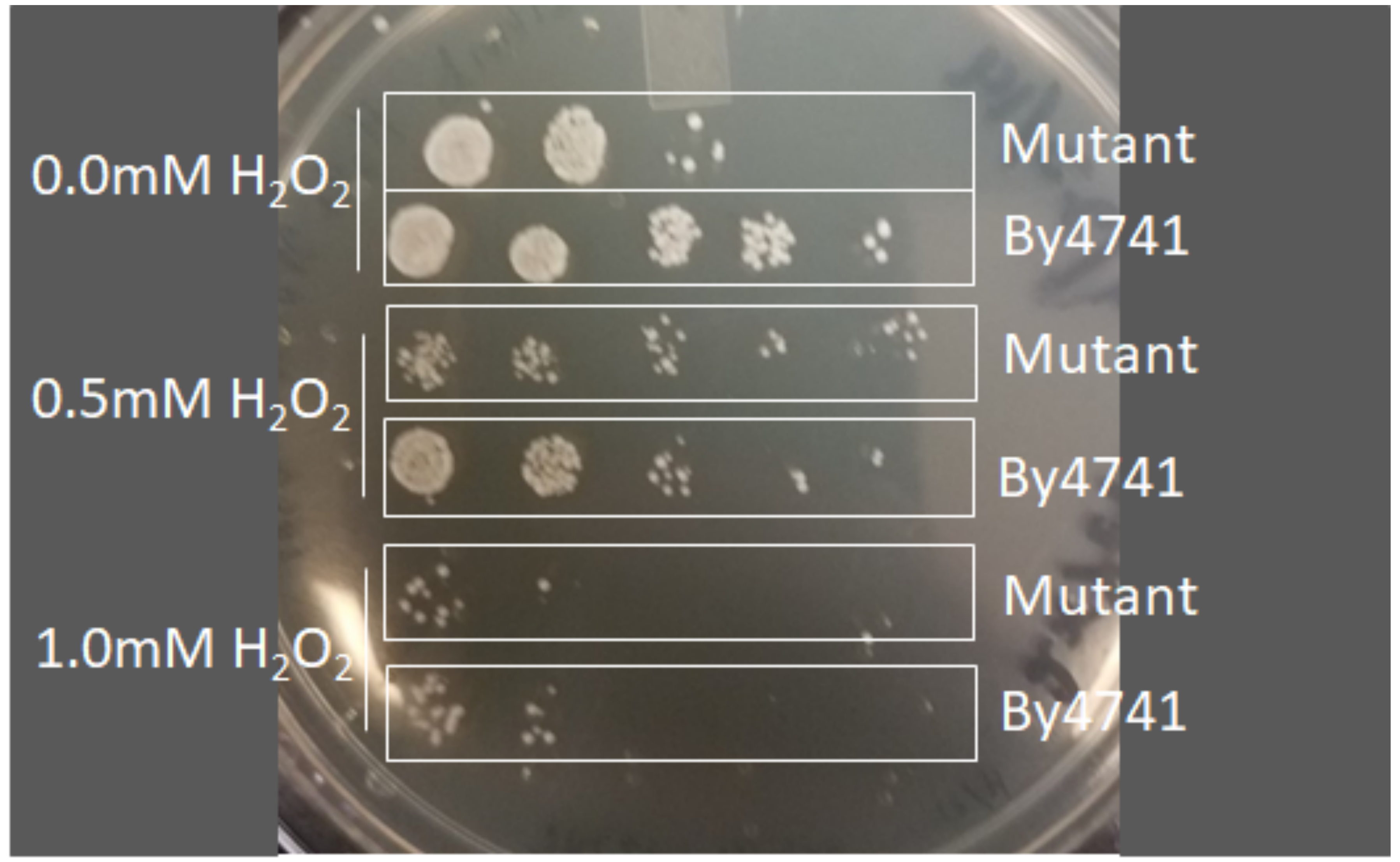

Figure 3: Oxidative Stress Test of Wild Type and *YIL055C* Deletion Mutants. Wild type (*BY4741*) Yeast were plated alongside *YIL055C* deletion mutant yeast on YPD plates. The Wild type and the mutant yeast were both incubated for one hour in H_2O_2. The concentration of H_2O_2 was increased as follows: 0.0 mM, 0.5 mM, 1.0 mM, 2.0 mM, 4.0 mM. The second plate that contained the 2.0 mM and 4.0 mM concentrations was omitted as it had no growth. They were then plated and left to grow for about a week. The above image shows one of two plates, this plate containing the most growth and the lowest concentrations of H_2O_2. The concentrations of H_2O_2 are displayed on the figure. The wild type yeast grew better in oxidative stress, thus demonstrating that the deletion of *YIL055C* does impact resistance to oxidative stress of Yeast organisms.

Cell Localization

By imaging cellular components of interest using MtioTracker™ Red and using GFP tagged YIL055C mutants, the images suggest that YIL055Cp does not localize to the Mitochondria. Comparing the image to other GFP labeled standards suggests an ER localization.

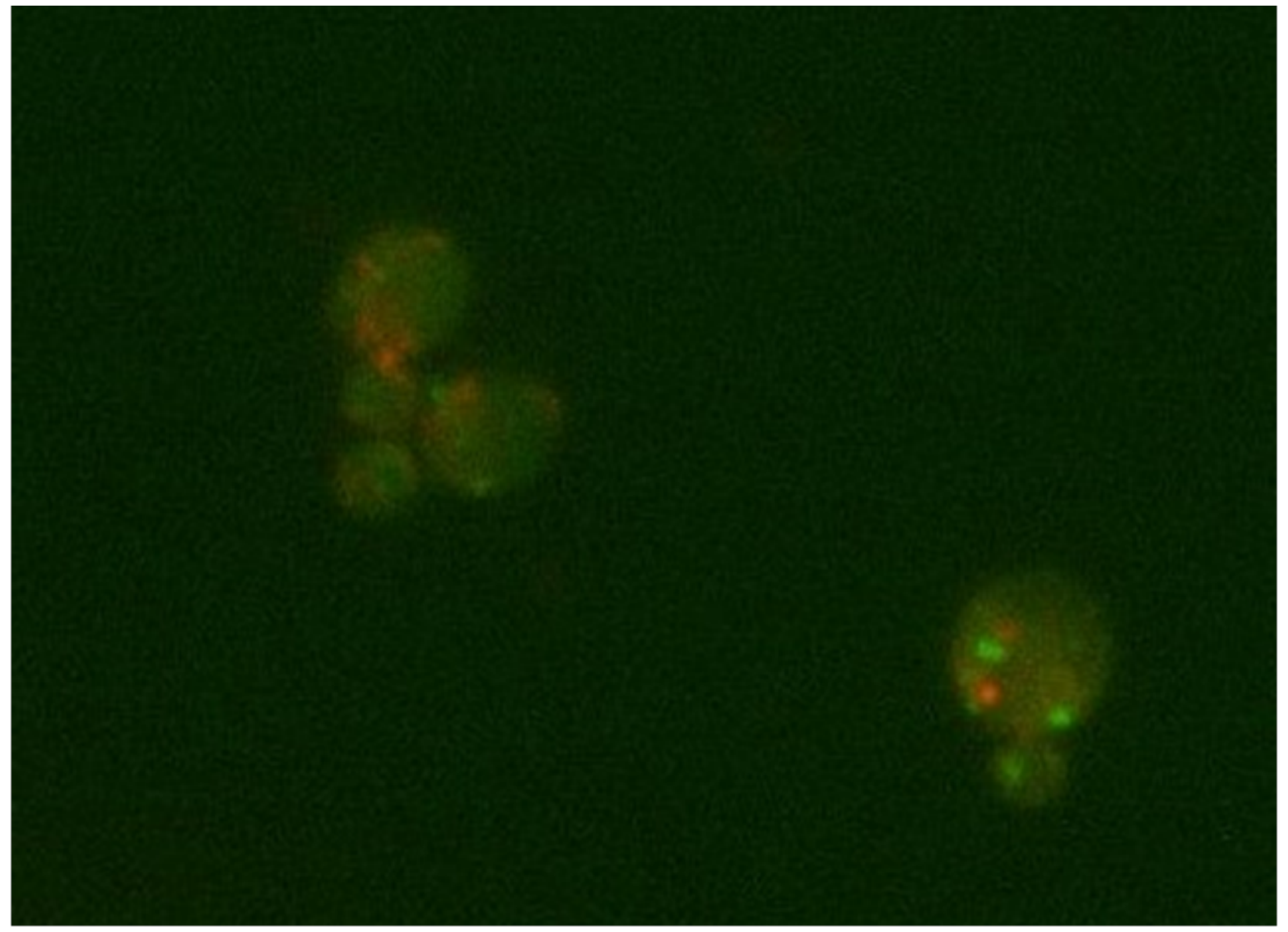

Figure 5: Image of Dual GFP and Mitotracker Red Microscopy. The above image shows the results of microscopy imaging to determine if YIL055Cp localizes to the Mitochondria. In the right cell, it is visible that the Mitochondria (colored red) and Yil055C protein (colored green) are in separate areas of the cell, but that YIL055C is contained in some other organelle as evidenced by its tight clustering.

Conclusion:

In conclusion, YIL055C seems to be an ER localized protein that assists in oxidative stress response. Which, notably, is in competition with the Sacaramyces Genome Database, which states that YIL055Cp is localized to the mitochondria. Further testing is required for more complete knowledge of the gene and its functions.

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