Fluorescent Detection of Reactive Oxygen Species in *Saccharomyces cerevisiae* Applied to Chronological Lifespan

Kelly Schultz, Dr. Megan Bestwick

Department of Chemistry, Linfield College, McMinnville, Oregon 97128

Introduction

During the course of normal aerobic metabolism, cells are exposed to a wide range of reactive oxygen species, such as the superoxide anion, hydrogen peroxide, the hydroxyl radical. These reactive oxygen species, or ROS, are highly reactive metabolites of oxygen and can damage a wide range of macromolecules in the cell (nucleic acids, proteins, and lipids) and can often times lead to cell death[1].

Normally, molecular oxygen is fairly unreactive and harmless in its' ground state. However, it is able to undergo partial reduction from electrons leaked from the electron transport chain to form both the superoxide anion and hydrogen peroxide, both of which can react further to form the dangerously reactive hydroxyl radical[2]. In order to combat the toxic and potentially deadly effects of ROS, cells are equipped with various antioxidant defense mechanisms. These mechanisms include enzymes like superoxide dismutase 1. When oxidative stress does occur, it is because a portion of ROS has overcome the host defenses like superoxide dismutase 1, resulting in oxidative damage to the cells.[2]

It's important to understand that superoxide dismutase 1 is primarily a cytosolic detoxifying enzyme that requires both copper and zinc cofactors to initiate and facilitate the conversion of the superoxide anion into hydrogen peroxide and then eventually, to water.

The overall objective of this research is to observe the abundance of different reactive oxygen species by applying various biochemical fluorescent staining assays to the chronological lifespan of the yeast, *Saccharomyces cerevisiae.*

Materials and Methods

- *S. cerevisiae* of superoxide dismutase 1 deletion strain (ΔSOD1), which was expected to have ROS sensitivity and a Wild Type (genetically the same, but no *SOD1* deletion), which was expected to have ROS resistance. Both were of the BY4743 strains .
- Cultures were inoculated (OD_{600}=0.5) in full YPD media and grown for 24hr.
- Experimental cultures were treated with 100 µL DHE+PBS stock solution (5 µL DHE, 995 µL PBS) and H_2O_2 (3 µL , 1mM).
- Fluorimeter was used to measure parameters of fluorescence at an emission range of 300-700 nm and an excitation of 405 nm.
- Dihydroethidium (DHE) was the biochemical dye used to quantitate levels of different reactive oxygen species produced by the yeast strains because it was the most specific and least problematic, as it is retained well by *S. cerevisiae*.

Literature Cited

[1]Dikalov, S. and Harrison, D. (2014). Methods for Detection of Mitochondrial and Cellular Reactive Oxygen Species. Antioxidants & Redox Signaling, 20(2), pp.372-382.

[2]Stowe, D. and Camara, A. (2009). Mitochondrial Reactive Oxygen Species Production in Excitable Cells: Modulators of Mitochondrial and Cell Function. Antioxidants & Redox Signaling, 11(6), pp.1373-1414.

[3]Friedman, N. The Friedman Lab Chronicles http://nirfriedmanlab.blogspot.com/2011/01/growing-yeasts-robotically.html (accessed Aug 5, 2017).

Acknowledgements

The Murdock College Research Program for Natural Sciences
The National Science Foundation
The Linfield Chemistry Department
Wendell L. Foote Fund

Further Information

Please contact Megan Bestwick at mbestwic@linfield.edu

Table 1: Intensity of fluorescence in WT and *ΔSOD1*.

Yeast Strain Observed at Ex. 405	Intensity	Normalized to WT
ΔSOD1+ DHE	3058	1.11
ΔSOD1- DHE	2879.5	1.04
WT + DHE	3222.5	1.16
WT - DHE	2772.1	1

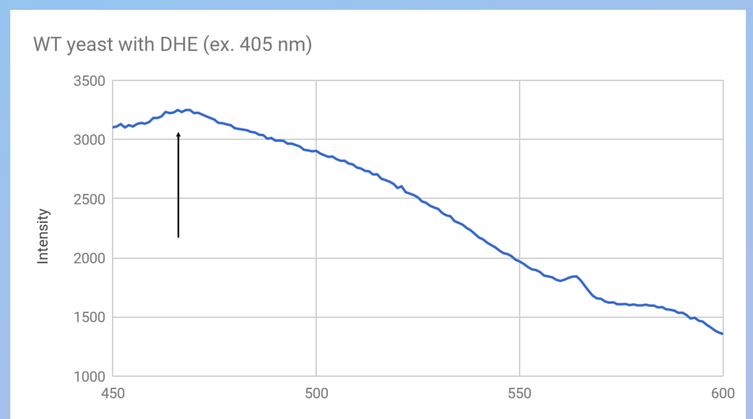

Figure 1: Fluorescence intensity in tracking H_2O_2 WT (+) DHE.

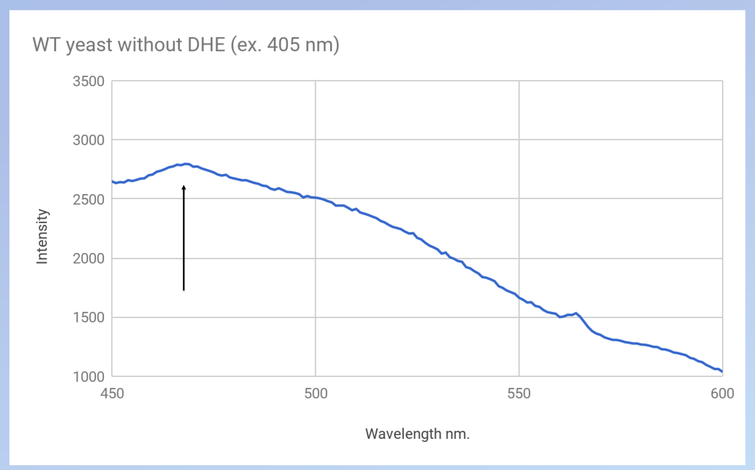

Figure 2: Fluorescence intensity in tracking H_2O_2 WT (-) DHE.

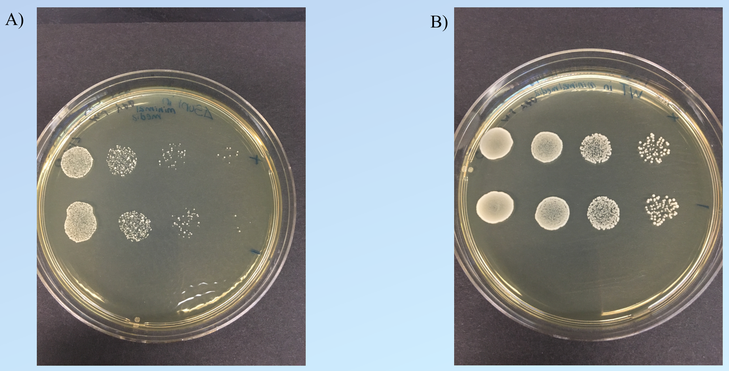

Figure 4: Drop test illustrating *ΔSOD1* (A) vs. WT (B) growth after H_2O_2 treatment (2 hrs.).

Results and Conclusions

Table 1 shows the intensity of fluorescence when the excitation was 405 nm and when the emission was 470 nm. There is a distinguishable difference in observable fluorescence between the WT strain treated with DHE and the WT strain not treated with DHE. However, we will need to do more trials to determine if this is a significant difference that can be reproduced. Values were normalized to the untreated WT strain, so that results could be more easily compared. When looking to the *ΔSOD1* strain, however, our results were not what were predicted. As exemplified by Table 1, there was more fluorescence observed overall in the wild type than in the *ΔSOD1*with DHE. It would be expected that a yeast strain deleted for *SOD1* would display more reactive oxygen species due to the lack of the detoxifying enzyme, but here we see greater fluorescence in the WT strains (indicating more ROS). It is clear that this data must be developed and reproduced further so that it can be understand as to why more ROS was being produced in the WT strain than in the *ΔSOD1*strain.

The results from the fluorimeter, provided us with solid preliminary data about DHE and how it reacts with Wild Type yeast. Even though there was some background in the trials, we can compare the Wild Type yeast strains quite well and can see that there is a greater intensity of fluorescence in the strain treated with DHE (Fig. 2) compared to the strain that wasn't (Fig.1). Hence, there is reason to believe that reactive oxygen species was able to be tracked using DHE.

Future Directions

- Future research will focus on obtaining more replicable results and establishing a greater difference between fluorescence emitted from the WT yeast with and without DHE.
- The discrepancies in the ROS presence in the *ΔSOD1* strains will also be further investigated by integrating the use of various positive controls such as respiratory chain inhibitors (Antimycin A and Oligomycin) in order to obtain replicable and distinguished results that will allow for the acquisition of a statistically significant, quantifiable difference in fluorescence between WT and *ΔSOD1* cultures.
- Treatment of cultures with fluorescent cell stains like Amplex® Red to detect extracellular hydrogen peroxide (H_2O_2) activity.
- Capture images via microscopy of reactive oxygen species in live yeast at various points during chronological lifespan. The grey bars in Figure 3 are indicative of the proposed time points.

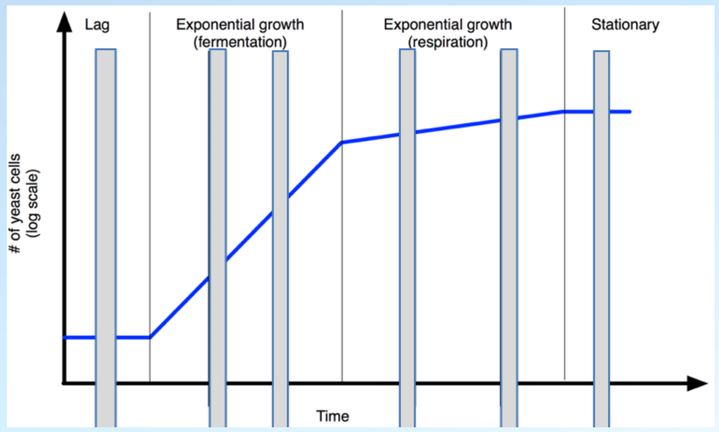

Figure 3: Typical yeast growth curve illustrating the time segments at which data on ROS levels will be collected[3].