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# Role of glutathione synthesis and recycling on fermentation efficiency and flavor compound production

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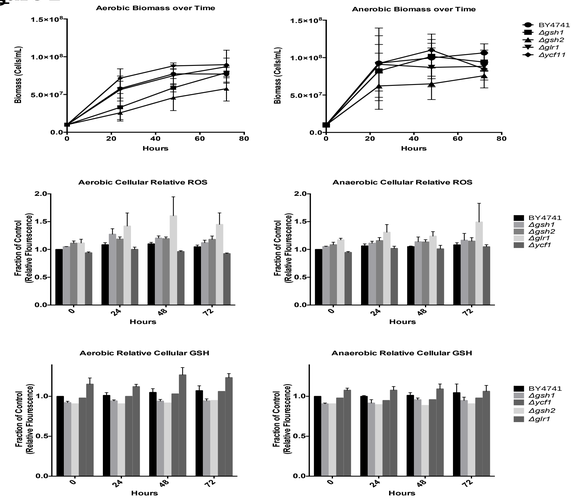
## Abstract

Over the last 5-10 years a number of groups interested in decreasing free radicals during the fermentation and bottling processes of beers and wines, have examined mechanisms to increase GSH content and excretion by yeast during the fermentation process. These studies have utilized classical and modern genetics to increase GSH content via increasing the GSH synthesis proteins Gsh1p and Gsh2p (Figure 1). The initial studies indicate that increasing yeast GSH cellular content and GSH excretion does increase the antioxidant capacity of the must and wort while also increasing the stability of beer and wine flavor post bottling. Further, a recent study published in 2014 examining Gpx1p and catalase (Cttp1p) mediated protection against oxidative stress support the role of glutathione as an important protective antioxidant in yeast during fermentation. Elevated levels and activity of Gpx1p and Ctt1p contribute to elevated cellular and extracellular GSH. Together these studies suggest an important role for the antioxidant glutathione based system in protecting yeast from oxidative stress during fermentation and for a role for GSH as a natural preservative that protects the beverages from oxidation. However, it is important to note that GSH is in equilibrium with GSSG and that this delicate balance is maintained via a complex multi-protein system containing the GSH synthesis proteins, Gsh1p and Gsh2p, glutathione reductase (Glr1p), and glutathione utilizing and linked proteins such as glutathione peroxidase (Gpx1p, Gpx2p, and Gpx3p), Ctt1p, and superoxide dismutase (Sod1p and Sod2p). To date no lab has examined how these systems work together to regulate oxidative stress during fermentation and regulate oxidation in bottled beer and wine. Our lab has utilized classical genetic and biochemical approaches to monitored oxidative stress, relative cellular GSH levels, and biomass. Ultimately, we hope that by exposing yeast brewing strains to an oxidant induce, our lab will selectively induce genes involved in GSH synthesis and recycling for use in the brewing industry .

## Methods

➢Wildtype *Saccharomyces cerevisiae* lab strain (BY4741) and four mutant strains *(Δgsh1, Δgsh2, Δglr1 and Δycf1*), were grown on standard YB agar under incubated conditions at 30ºC for 48-72 hours.Five flasks were set up for anaerobic experimentation at a concentration of $1.0x10^7$ cells/mL in 25 mL of DME for aerobic and 125 mL for anaerobic conditions were inoculated with a single colony of each strain..Biomass, relative ROS was measured using DCFDA vital and relative GSH cellular levels was measured using GSH cellular stain (Ursa chemicals) using a fluorometric cellometer-X2 (Nexcelom) at 0, 24, 48 and 72 hours for both aerobic and anaerobic samples.

**Figure 2**




## Results

➢Deletion of *Δycf1*, the protein responsible for GSH transport into the vacuole, results in increased cellular GSH, decreased ROS, and increased Biomass under aerobic conditions as compared to control. Growth under anaerobic conditions does not result in an increase of cellular GSH and Biomass nor decreased ROS (Figure 2, middle panels).
➢Deletion of GSH synthesis genes *Δgsh1* and *Δgsh2* results in a significant decrease in cellular GSH, increased ROS, and decreased Biomass under aerobic conditions as compared to control. However under anaerobic conditions only *Δgsh2* results in decrease Biomass (ROS (Figure 2, top panels).
➢Deletion of the GSH recylcing gene *Δglr1* results in a increased ROS under aerobic and anaerobic conditions but no difference in cellular GSH levels or Biomass as compared to control ROS (Figure 2, bottom panels).

## Conclusion

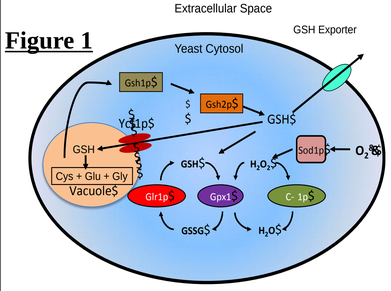
- GSH cellular levels play an important role in regulating cellular ROS during aerobic and anaerobic yeast growth.
- Changes in cellular GSH and ROS play a role in regulating Biomass.
- Ssuggests that the glutathione synthesis and recycling pathways are important in regulating fermentation efficiency.
- Future studies we will examine the rate of sugar consumption and alcohol consumption, as well as production of important flavor chemicals during fermentation in all five mutants.

## Impact

Non-GMO yeast that have increase cellular GSH may prove an important tool to producing Beer and Wine more efficiently in the future. Increased efficiency of fermentation will decrease production time and thereby increase profitability.

## GSH Synthesis & Recycling

**Figure 1**

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