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2017 ASBC Annual Meeting

Role of Glutathione in Yeast Growth and Fermentation for Beer and Wine Production

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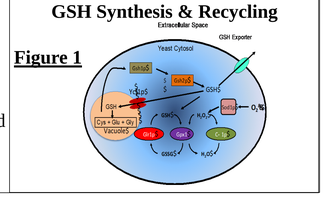
Abstract

To date no lab has examined how the glutathione (GSH), synthesis, recycling, and GSH stress response systems work together to regulate oxidative stress during fermentation and regulate oxidation in bottled beer and wine. Our lab has utilized classical genetic approaches, molecular biology, and the yeast deletion collection to increase GSH content of a standard laboratory strain of *Saccharomyces cerevisiae* (BY4741 background). We have measured and compared fermentation efficiency in each of the deletion, control, and a number of beer brewing strains. For all strains biomass, relative oxidative stress, and cellular GSH levels was measured. Oxidative stress was measured as a function of Dichlorofluorescein-diacetate (DCFDA) fluorescence, a measure of general reactive oxygen species (ROS). The DCFDA results were then compared to measured levels of GSH. To investigate the role of GSH in traditional brewing strains, we selected three commercially available brewing strains, from White Labs, American Ale, Oktoberfest, and *Brettanomyces bruxellensus.* For each brewing strain we measured biomass, relative ROS via DCFDA fluorescence, and cellular GSH content as described above. To determine if increased GSH results in increased fermentation efficiency we treated each of the three strains with Molybdenum to select for strains with increased cellular GSH levels.

Introduction

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 Glutathione (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 glutathione synthase 1 and 2 (Gsh1p and Gsh2p). 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 glutathione peroxidase 1 (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. 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).

Figure 1



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 aerobic experimentation at a concentration of $1.0x10^7$ cells/mL in 25 mL of DME for aerobic and 125 mL for aerobic 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.
- For analysis of standard brewing strains (WLP060 – American Ale, WLP820 – Oktoberfest Lager, and WLP650 – *Brettanomyces bruxellensis*) experiments measuring biomass, DCFDA, and GSH cellular content for aerobic fermentation as described above.
- To select for brewing strains that have increased GSH synthesis and hence cellular concentrations, we plated WLP060 – American Ale, WLP820 – Oktoberfest Lager, and WLP650 – *Brettanomyces bruxellensis* on YPD plates containing 10mM Ammonium Molybdate.

Figure 2

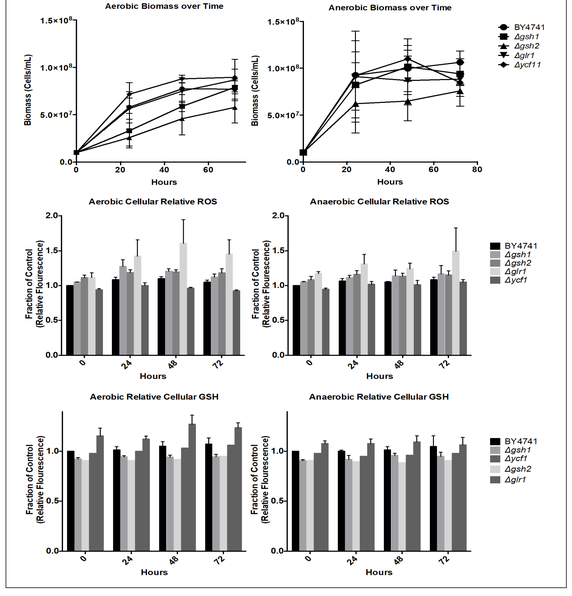
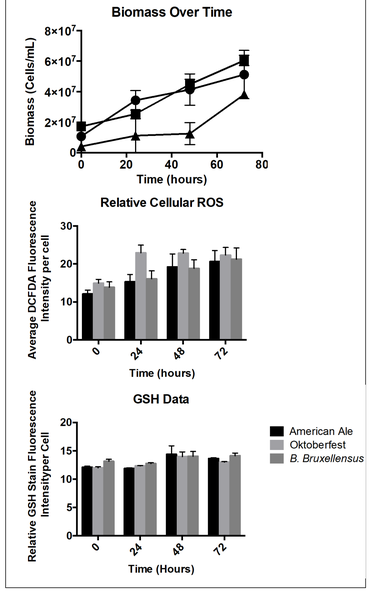
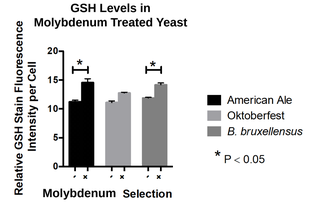


Figure 3



Results

Figure 4



- Deletion of *Δycf1* results in increased cellular GSH, decreased ROS, and increased Biomass under aerobic conditions as compared to control.
- Deletion of GSH synthesis genes *Δgsh1* and *Δgsh2* results in a significant decrease in cellular GSH, increased ROS, and decreased Biomass under aerobic and anaerobic conditions as compared to control (Figure 2).
- *Brettanomyces Bruxellensis* grows slower than either the American Ale Blend or the Oktoberfest Lager strain as expected under normal conditions (Figure 3).
- No significance difference in relative cellular ROS (DCFDA) or GSH celllar content was found between *Brettanomyces bruxellensis*, American Ale Blend, or Oktoberfest Lager yeast strains (Figure 3).
- Selection of American Ale, Oktoberfest Lager, and *B. brunxellensis* on Molybdenum containing plates increased cellular GSH (Figure 4)

Conclusion

- GSH cellular levels play an important role in regulating cellular growth and cellular ROS during aerobic and anaerobic yeast growth in standard laboratory yeast strains.
- GSH cellular content and Relative cellular ROS was similar in three standard brewing strains examined
- Suggests that standard brewing strains may have adapted to have optimized their cellular GSH levels as to regulate fluctuations in cellular ROS under the conditions measured.
- Created a non-GMO brewing strain with increased GSH cellular levels. via selection with Molybdenum. In the future we will further characterize the yeast growth efficiency, fermentation efficiency, cellular ROS, and long term oxidation in finished products.

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