203

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Generation of Novel Strains of Low-Alcohol Producing Brewing Yeast by Selection for Alcohol Dehydrogenase Defective Mutants

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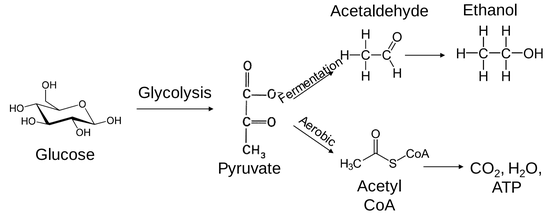
Introduction

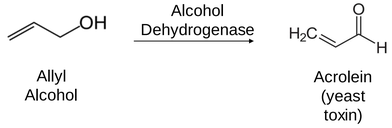
Recently there has been movement with "session beers" becoming a rapidly growing craft brewing sector. A primary challenge faced in the production of a quality session beer is maintaining a strong, unique, and well balanced flavor and aroma profile while keeping the malt content low, thus resulting in a flavorful yet only moderately alcoholic beer. Yeast can participate in both aerobic and anaerobic metabolic pathways. Lack of available oxygen forces yeast to ferment sugars, creating ethanol. In the presence of oxygen, aerobic pathways will result in the production of CO_2, H_2O, and energy.



The purpose of this project is to create a strain of brewing yeast that will favor aerobic metabolism over anaerobic fermentation, thus producing a beer of similar flavor and aroma profile, but with lower than normal ethanol concentration.

Methods

Approximately 1.0 x 10^8 cells of *Saccharomyces cerevisiae* (Wyeast 1056 yeast) were mutated using ethyl methyl sulfonate and plated on nutrient rich agar plates containing 8mM allyl alcohol. When metabolized by alcohol dehydrogenase, allyl alcohol produces a toxin that will kill the yeast.



Yeast with a defective alcohol dehydrogenase enzyme are able to survive on the allyl alcohol plates due to an inability to metabolize allyl alcohol. Figure 1 below shows the growth comparison of mutant yeast on a regular nutrient rich agar plate versus a plate containing allyl alcohol.

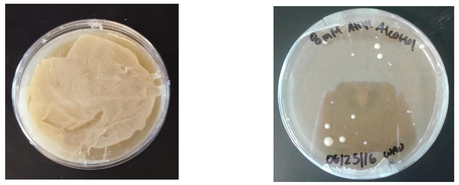


Figure 1: Comparison of growth of mutant yeast on a regular plate (left) versus a plate containing allyl alcohol (right). The plate to the left could be described as a "lawn", with no distinction between individual colonies. On the plate to the right, individual colonies can clearly be seen.

Individual surviving colonies are picked from the plates, inoculated in a 25 mL liquid culture, and are used for pilot fermentations. During aerobic trials, the fermentations were oxygenated with sterile air for 1 minute every 8 hours. Initial specific gravity and percent Brix measurements were recorded using an Anton Paar Density Meter and a refractometer. Once the fermentations had been completed, final specific gravity and percent Brix measurements were recorded. A final ethanol concentration measurement was taken using an Agilent Technologies 7890B gas chromatograph.

Results

Surviving colonies were inoculated into fresh wort and the progress of the fermentation was monitored in comparison to a control fermentation.

Figure 2: Sugar Concentration vs. Fermentation Time

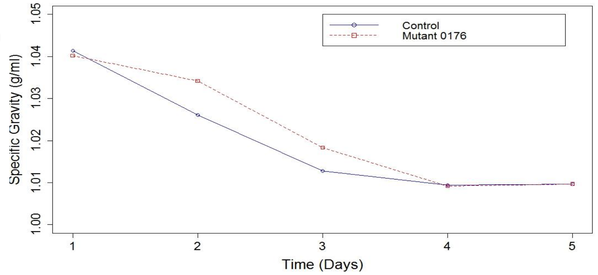


Figure 2: Fermentation progress of a control yeast versus a mutant yeast measured by specific gravity as a function of time. The clearly shows that the mutant yeast are able to metabolize sugar in a similar fashion to wild type yeast.

At the end of each pilot fermentation, the final ethanol concentration for the mutant yeast was measured and recorded. A control was included in each trial for comparison.

Figure 3: Final Alcohol Concentration Produced by Individual Mutant Strains

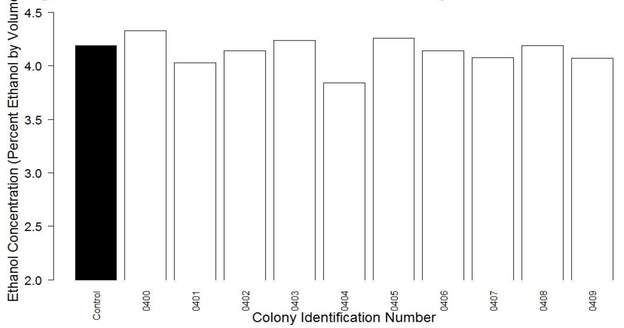
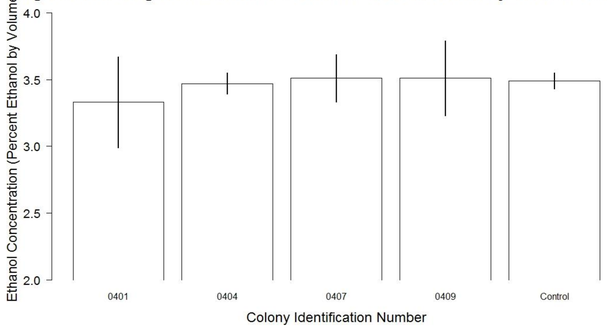


Figure 3: Final ethanol concentration in percent ethanol by volume for mutant yeast colonies. It is worth noting that all pilot fermentations reached a similar terminal specific gravity of 1.010 g/mL.(Note: y-axis scale altered to better depict differences)

A pilot fermentation was conducted in triplicate of 0401, 0404, 0407, and 0409 mutant strains, which showed decreased ethanol production. A control was also run in triplicate.

Figure 4: Average Final Alcohol Concentration Produced by Selected Mutant



Conclusions

- The mutation and selection protocol was successful at selecting mutants with limited ADH-1 enzyme activity. These mutants are metabolically healthy in comparison to the control yeast in the presence of allyl alcohol.
- Decreases in ABV of approximately 0.45% have been recorded, representing a 10% relative decrease in ethanol production.
- Although decreases in ethanol production have been observed, they were expected to be greater. A possible explanation of this observation is the presence of multiple isoforms of alcohol dehydrogenase, only some of which have been damaged by mutation.
- Since allyl alcohol has variable mitochondrial membrane permeability (Saliola et al. 2006), an isoform of ADH found in the mitochondria (Crichton et al. 2007; De Smidt et al. 2008) may not be affected by our screening protocol but would still allow production of ethanol.
- Our selection method potentially selects mutants with reduced alcohol binding affinity. This would not then affect the acetaldehyde to ethanol reaction, allowing normal ethanol production.

Future Studies

- Further refinement of the selection protocol
- Examinations of The Crabtree Effect and Pasteur Effect in mutant yeast to see if the balance of aerobic metabolism versus fermentation can be altered.

References and Acknowledgements

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