

Genetic Profiling of Twelve Commonly Used Brewing Yeast Strains: A Productive Collaboration Between Beer and Undergraduates

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Beer and Undergrads?!

- Educational value of inquiry-based and authentic research
 - Skill-building
 - Tractable
 - Engaging and relatable
- Yeast genetics and beer!
- Formulating the research question



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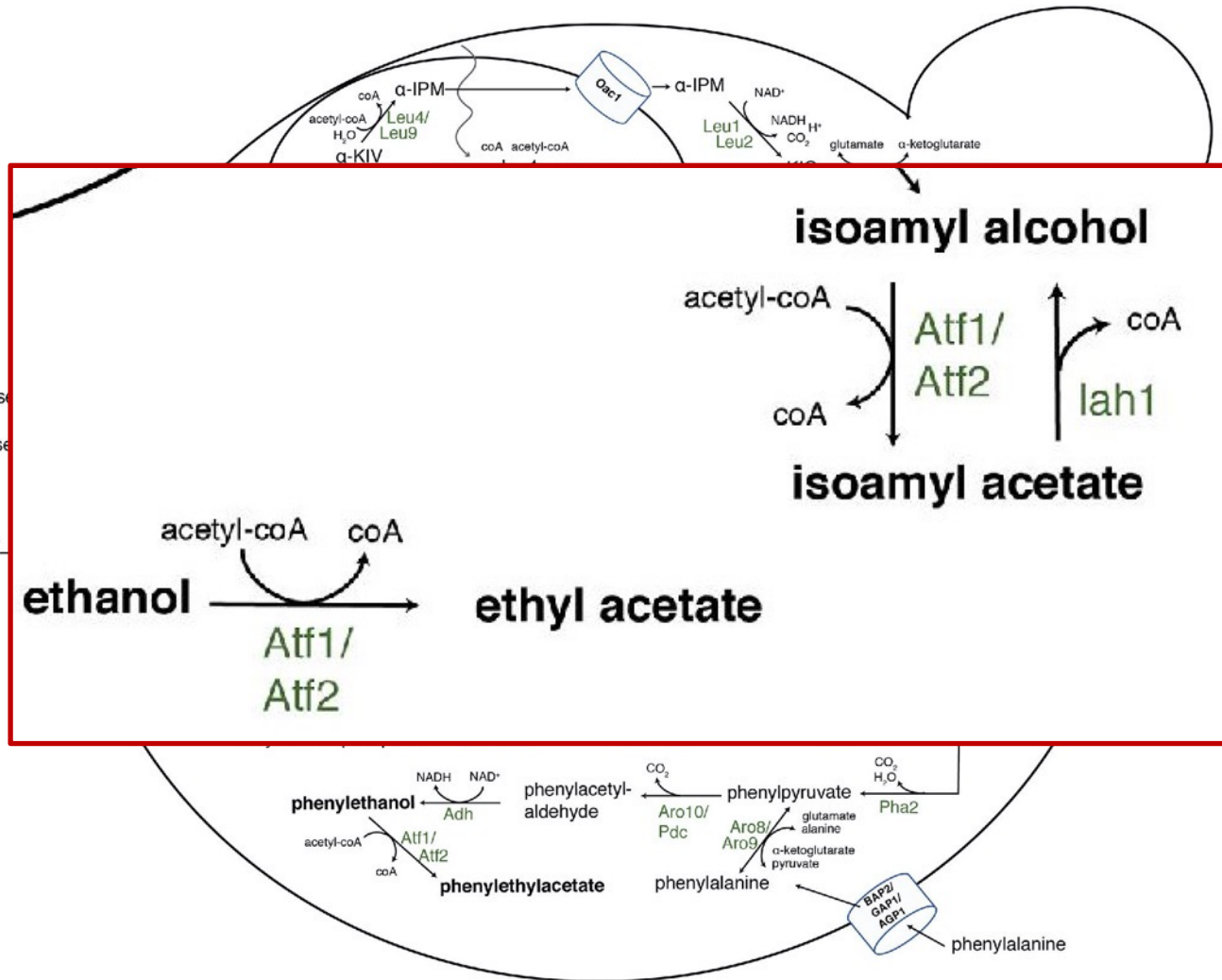


The Research Question Inspired by a Local Brewery...



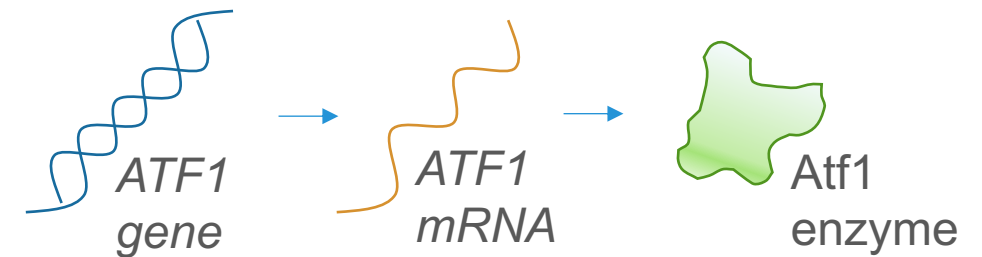
- High Point Brewing Company, founded by Greg Zaccardi
- Re-pitching Hefeweizen yeast
 - Isoamyl acetate drop-off after a few generations

Overview of Biochemical Pathways Driving the Formation of Aroma Producing Esters



During fermentation, yeast metabolism produces aromatic compounds.

- Isoamyl acetate (banana) & ethyl acetate (fruity to "solvent-like")
- *ATF1* & *ATF2* (alcohol acetyl-coA transferase), and *IAH1* (isoamyl acetate-hydrolyzing esterase) code for enzymes catalyzing the reactions



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<https://doi.org/10.1093/femsre/fuy041>

Strain Selection



White Labs Code	Yeast Strain Name
WLP001	California Ale
WLP007	Dry English
WLP029	German/Kolsch Ale
WLP090	San Diego Super
WLP300	Hefe Ale
WLP550	Belgian Ale
WLP400	Belgain Wit
WLP775	English Cider
WLP500	Monestary Ale
WLP565	Belgian Saison I Ale
WLP036	Dusseldorf Alt Ale
WLP644	Saccharomyces brux-like Trois

Selecting yeast strains with a wide range of aroma profiles

➤ *With a little help from my local homebrew store (Cask and Kettle)*



What are the genetic differences between strains, which give rise to the different aroma profiles?

- Are there allelic differences between strains for *ATF1*, *ATF2*, and *IAH1*?
- *Are there gene expression differences between strains for ATF1, ATF2, and IAH1?*



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Methodology



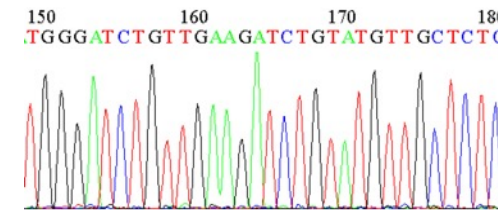
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Sequencing *ATF1*, *ATF2*, and *IAH1*

- Yeast strains cultured, harvested, and genomes extracted.
- Primers designed using database and bioinformatics software.



- Performed PCR for each gene.
- Sequenced *ATF1*, *ATF2*, and *IAH1* for each strain.



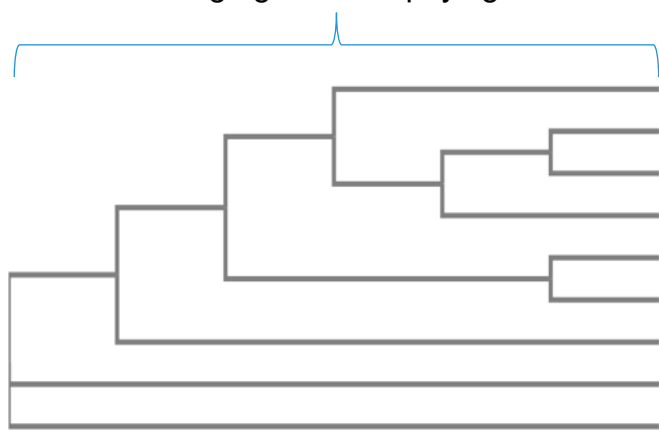
- Annotated each sequence and identified mutations.



Sequencing *ATF1*, *ATF2*, and *IAH1*

All *ATF1* mutations were synonymous (*Dusseldorf Alt* and *Belgian Ale* sequences omitted).

Clustal Omega generated phylogenetic trees



ATF2 Mutations

Strain	V64L	L156V	S289T	Q305E	V306G	C330W	E435Q	G530S
Belgian Wit	x	x	x	x			x	
Dry English	x	x	x	x			x	x
German/Kolsch	x	x	x	x			x	x
San Diego Super	x	x	x	x			x	x
S288C (reference genome)								
English Cider					x	x		
Saccharomyces brux-like Trois							x	x
Monestary Ale							x	x
Belgian Saison I							x	x

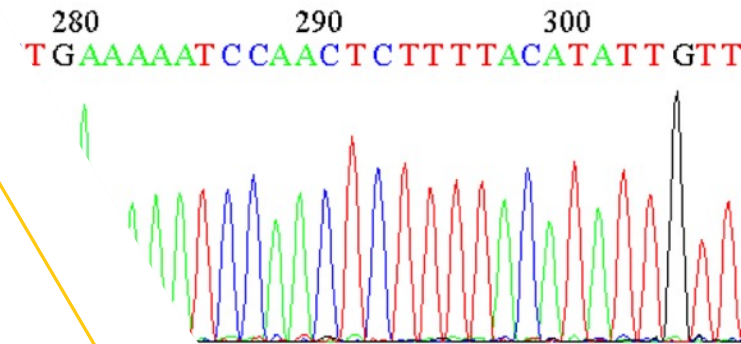
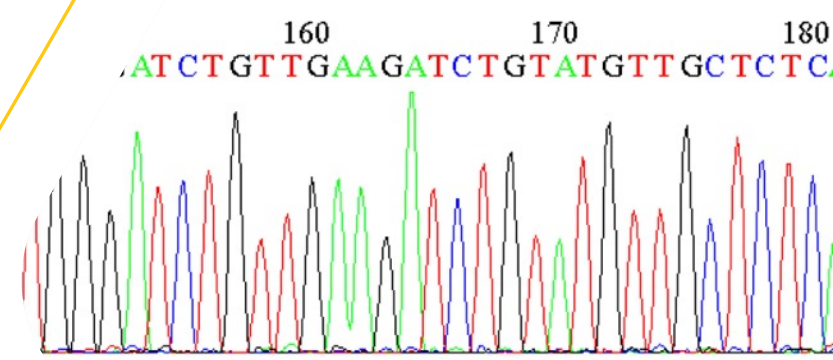


IAH1 Mutations

Strain	D11A	I23T	K163N
San Diego Super	x		x
S288C (reference genome)			
Belgian Wit		x	
Monestary Ale		x	x
Dry English		x	x
German/Kolsch		x	x

Sequencing *ATF1*, *ATF2*, and *IAH1*

- None of the strains sequenced showed any *ATF1* divergence, which could indicate its critical/desirable role that it plays during fermentation.
- Both *ATF2* and *IAH1* had a number of non-synonymous mutations between the strains; however, the functional consequences of these mutations require further investigation.
- There were no frameshift or non-sense mutations found that would have an obvious functional consequence on enzyme activity.





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Testing for Gene Expression – Experimental Design

Starter cultures inoculated and grown in wort O/N

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O/N
@ 20 °C

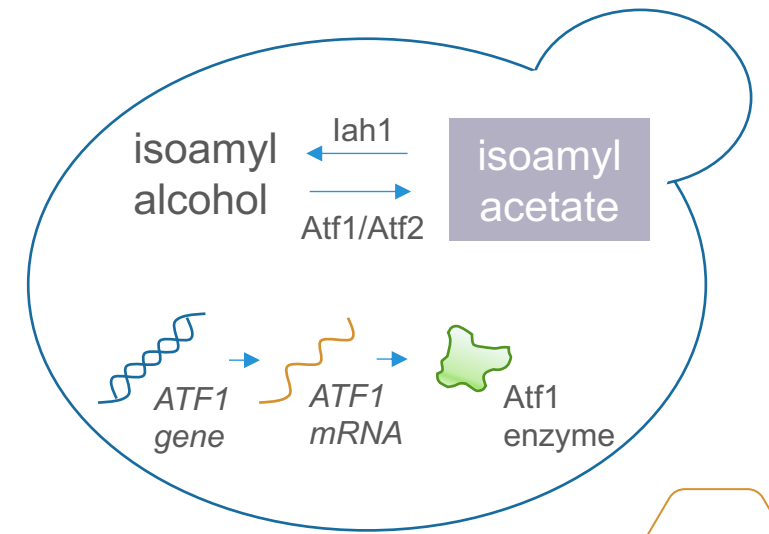
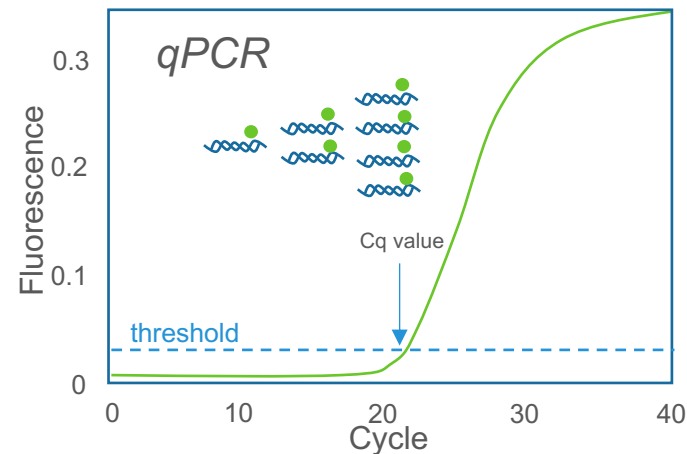
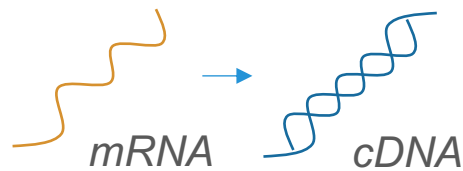
- Side-by-side small-batch fermenters inoculated w/ 9×10^6 cell/ml.
- 150 ml of unhopped 12 °P golden light DME.



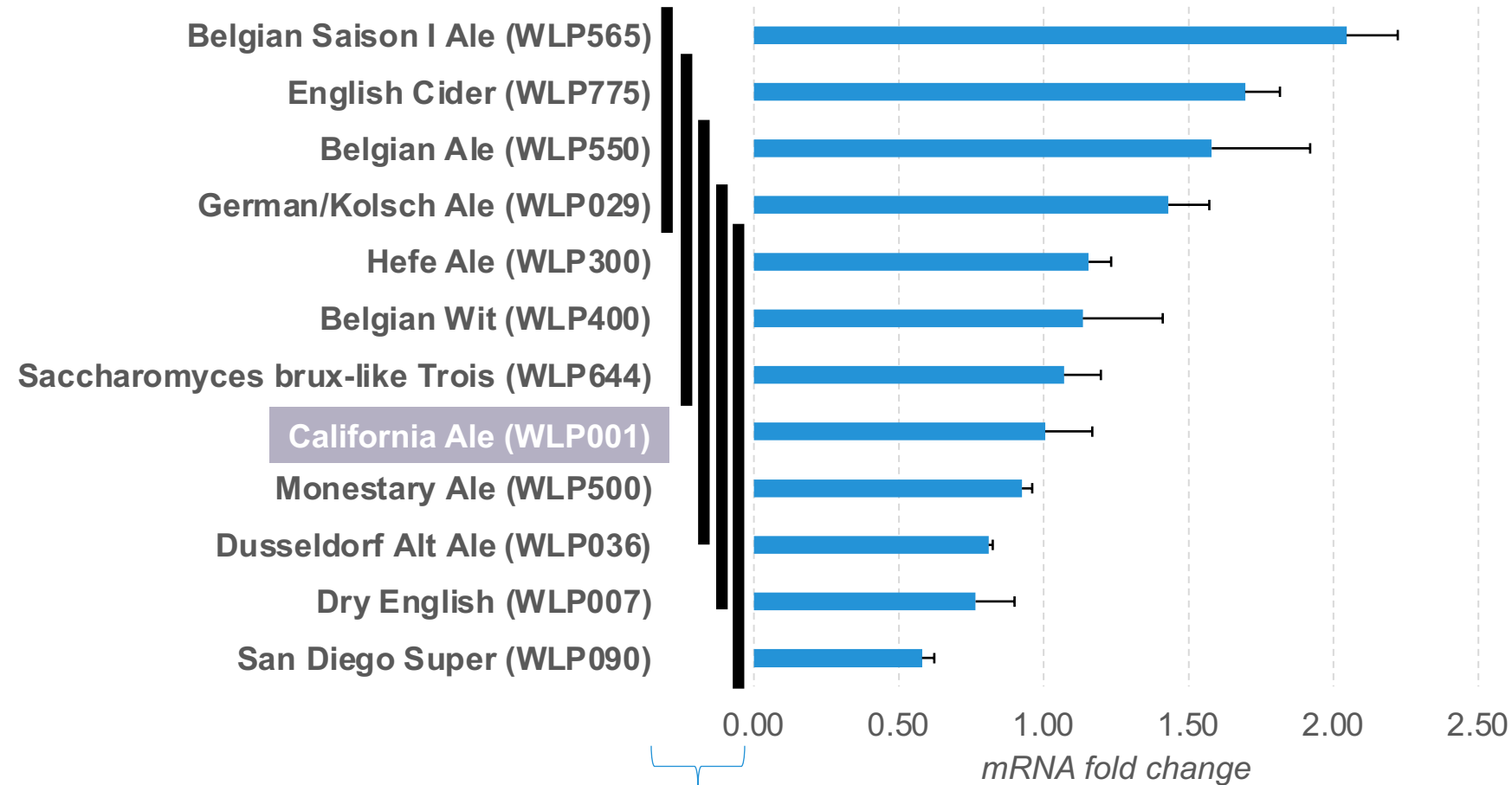
72 hours
@ 20 °C

Samples Collected during mid/late primary fermentation

- "Sensory Evaluation"
- Gravities measured
 - Attenuation ranged from 62%-83%
- Cells harvested and immediately processed for RNA extraction



Relative *ATF1* Gene Expression



Overlapping bars indicate no significance ($P > 0.05$)

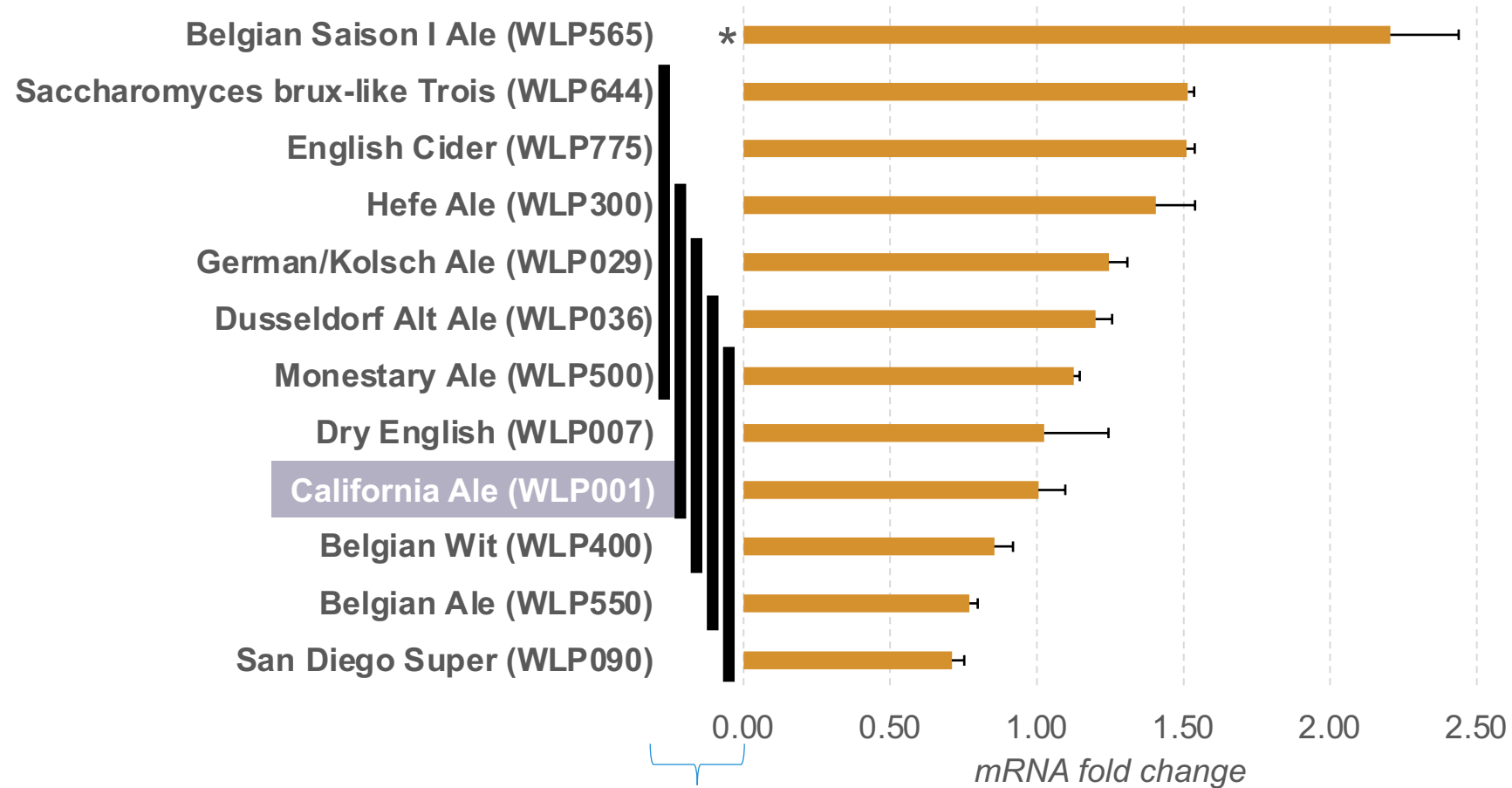
mRNA expression determined using $\Delta\Delta Cq$ method

- *ACT1* (reference gene)
- California Ale (WLP001) served as the control strain

Three biological replicates and two technical replicates

One-way ANOVA (Tukey-Kramer-test)

Relative ATF2 Gene Expression



Overlapping bars indicate no significance ($P > 0.05$)

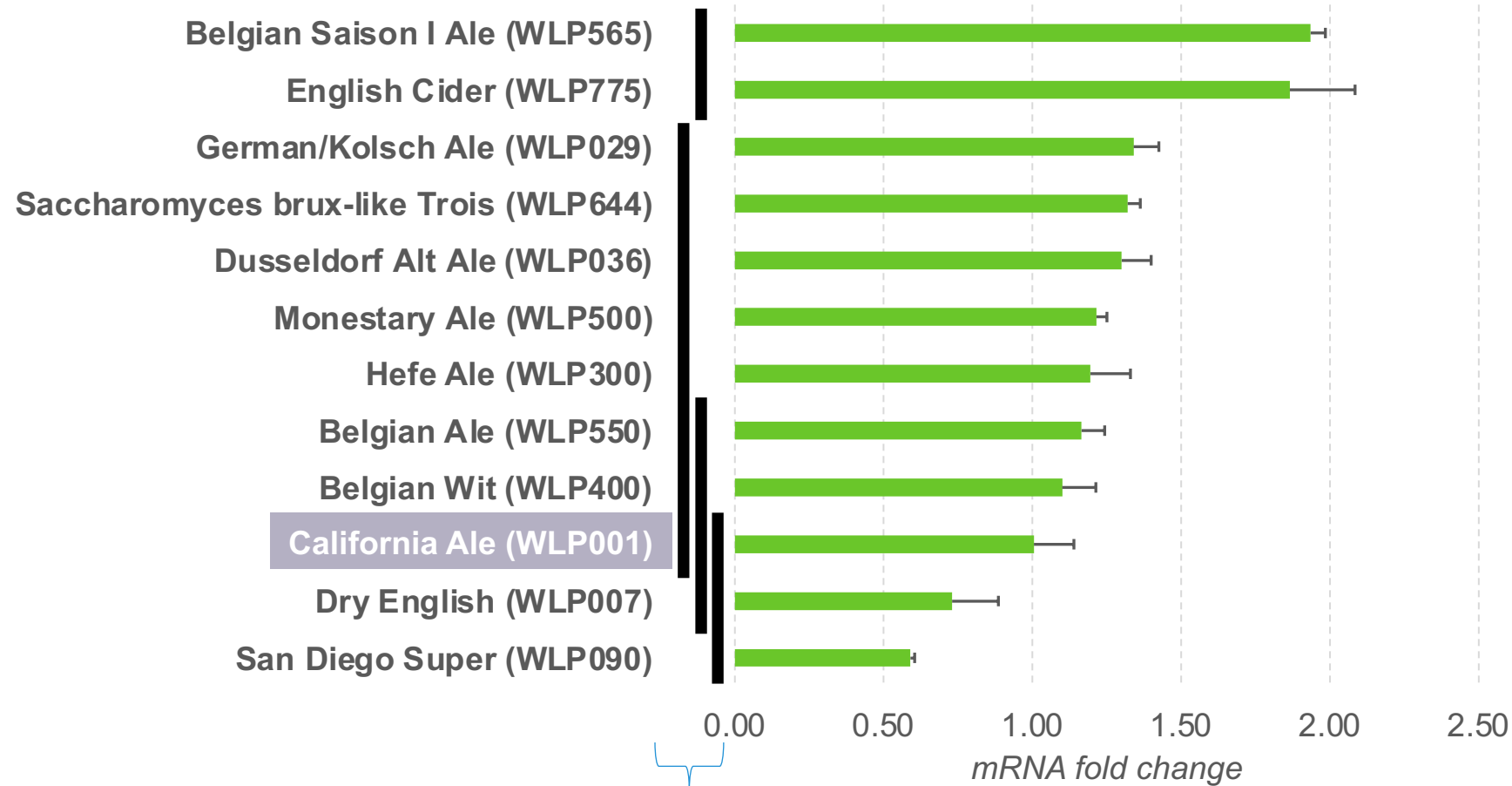
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Three biological replicates and two technical replicates

One-way ANOVA (Tukey-Kramer-test)

Relative *IAH1* Gene Expression



Overlapping bars indicate no significance ($P > 0.05$)

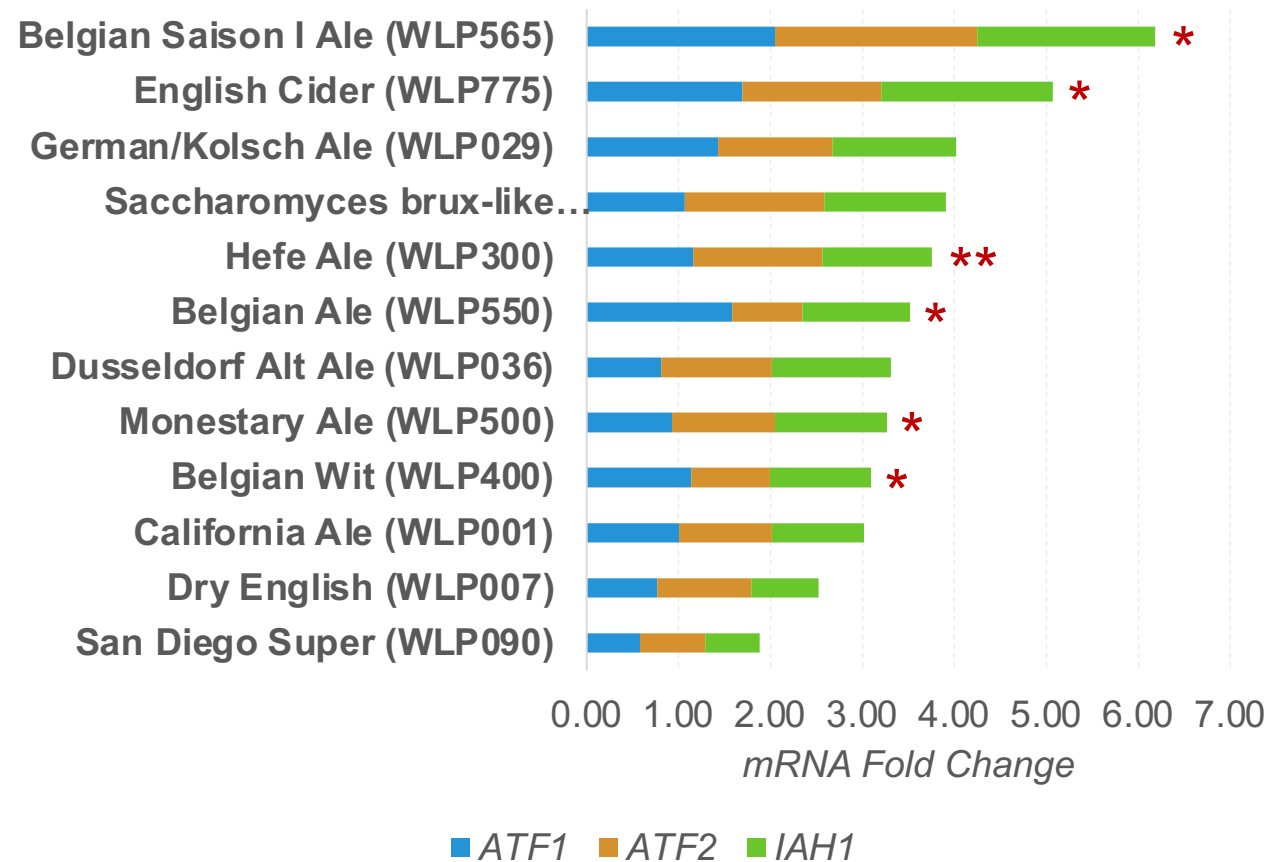
mRNA expression determined using $\Delta\Delta Cq$ method

- *ACT1* (reference gene)
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Three biological replicates and two technical replicates

One-way ANOVA (Tukey-Kramer-test)

Conclusions



There are notable differences in expression for *ATF1*, *ATF2*, and *IAH1* between yeast strains.

- Surprisingly, strains known to produce high isoamyl acetate levels/fruity notes were scattered.
 - Hefe Ale & Belgian varieties
- San Diego Super, a low ester producing strain, showed the lowest levels of expression for all genes.

Fermentation rates varied between strains and thus the timepoint for sample collection was not optimal for all strains.

Future Directions and Applications

Look at gene expression over a series of timepoints during fermentation.

- Some strains may express early while others later.
 - Inform brewers about which combination of strains to use.

Couple experiments with a quantitative assay for isoamyl acetate levels.

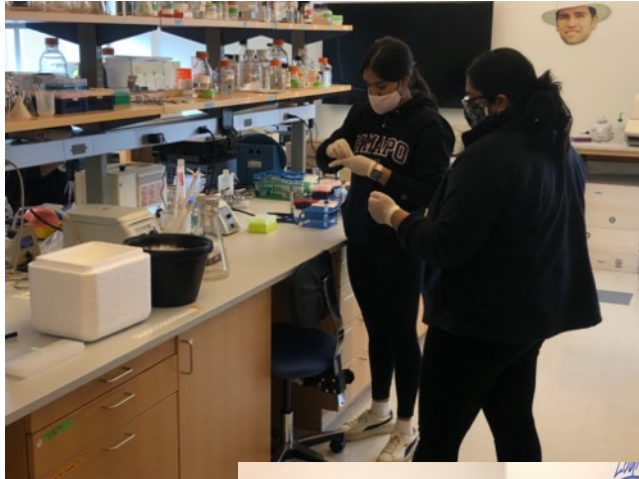
Examine the effect that varying temperatures have on gene expression.

Expand the genes and strains examined to get a broader spectrum.

- Generate a gene expression database.
 - Informative for using and generating new strains.

Conduct these experiments in collaboration with a brewery.

Acknowledgments



Students!

Introduction

... booming and lucrative business due to beer flavors (projected to be worth \$190 billion)

... motivated to improve their beer (select different clients)?

... *Saccharomyces cerevisiae* produces the alcohol and contributes to the overall beer flavor/aroma profile?

... undesirable aromatic compounds via metabolic pathways?

... have artificially selected for yeast strains & the traits they are looking for in their beer: ... genetic background of yeast remains characterized?

... genetics play in imparting flavor/aroma during "the brew"?

Experimental

1. Strains purchased from White Labs (yeast distributor).
2. Yeast grown on agar plates and as liquid cultures.
3. Yeast DNA isolated and extracted to analyze genes of interest.
4. Primers designed to direct DNA synthesis in prep for PCR.
5. To amplify the DNA and copy a specific portion of interest, polymerase chain reaction (PCR) was performed.
6. Gel electrophoresis performed to visualize PCR results.
7. Successful strains sent out for sequencing to determine the nucleotide sequence of DNA.
8. ABE (Bioinformatic Tool) used to analyze sequencing files for mutations.

Results

ATF1:

ATF2:

IAH1:

Acknowledgments

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