Effect of Acetyl Co-A Overexpression on Fatty Alcohol Production in *Saccharomyces cerevisiae*

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Introduction

- The fatty alcohols hexadeconal and octadeconal are long chain amphiphilic molecules widely used in the cosmetics and pharmaceuticals industries. Historically, the main source of these chemicals was oil harvested from heads of sperm whales; however, they occur rarely in nature.
- Fatty alcohols are currently synthesized from palm oil, which has escalated the amount of deforestation in Southeast Asia due to the establishment of palm plantations. This has led to a loss of biodiversity [1].
- The microorganism *Saccharomyces cerevisiae* is an easily genetically modifiable yeast strain ideal for scale up due to its extensive usage in large batch fermentation.
- Metabolic engineering and synthetic biology techniques have been used to modify the fatty acid biosynthesis pathway in *S. cerevisiae,* introducing exogenous genes to synthetically produce fatty alcohols [2].

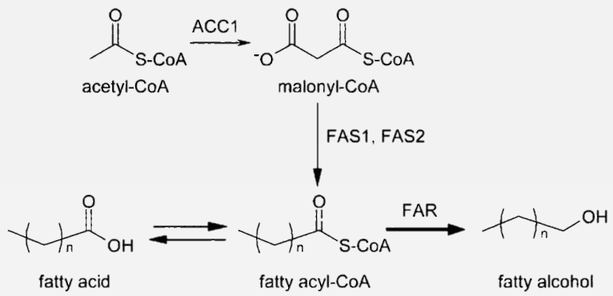

Figure 1. Basic pathway for production of fatty acids in *S. cerevisiae*. Introduction of an exogenous fatty acyl reductase (FAR) will result in the production of fatty alcohols.

Objective

Our primary purpose is to investigate the effects of overexpressing acetyl-CoA on overall yield of fatty alcohol production by using yeast as a model organism.

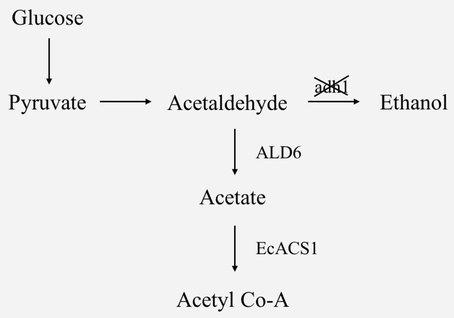

Figure 2. Pathway for acetyl – CoA synthesis with proposed genetic modifications to create the overexpression. The adh1 knockout and increased expression of the ALD6-EcACSI gene cassette should drive up acetyl-coA production.

Methods & Procedures

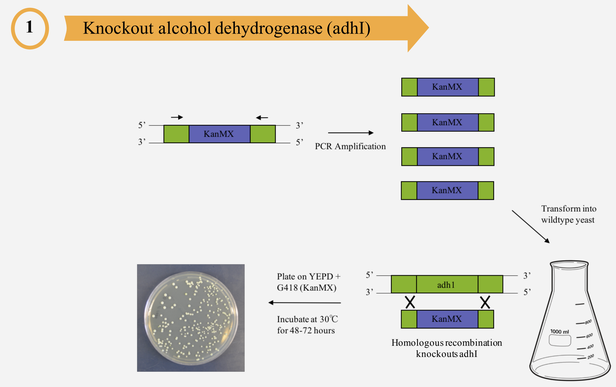

Figure 3. Workflow indicating creation of adh1 knockout.

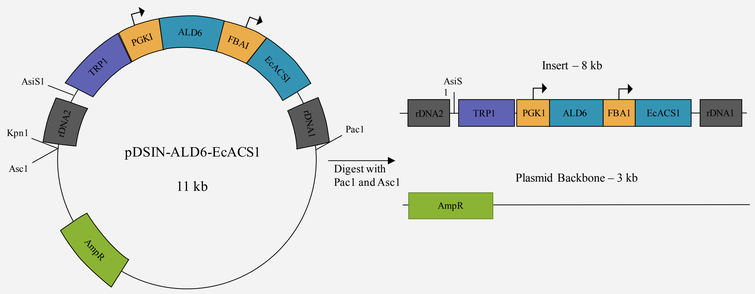

Figure 4. Restriction digest of plasmid containing ALD6 and *E. coli* EcACS1.

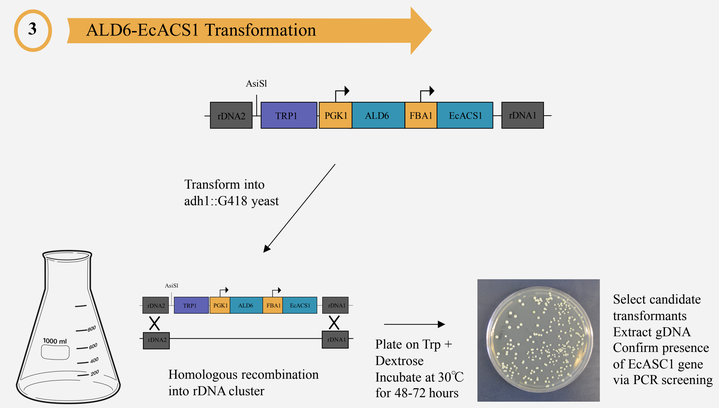

Figure 5. Workflow for integration of ALD6-EcACS1 overexpression cassette into *S. cerevisiae* rDNA clusters.

Results

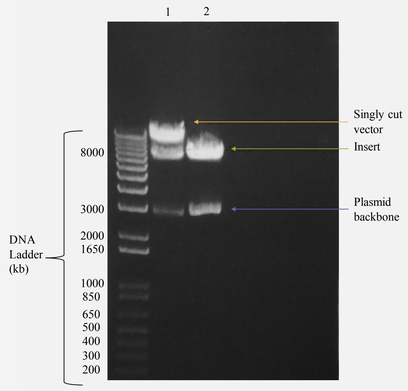

Figure 6. pDSIN-ALD6-EcACS1 Pac1/Asc1 restriction digest in lanes 1 and 2 resulting in the expected 8000 kilobase (kb) insert and a 3000 kb plasmid backbone. Digests, as well as ALD6-EcACS1 strains in figure 7, were screened through gel electrophoresis.

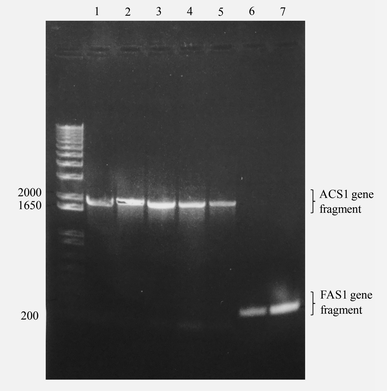

Figure 7. Polymerase Chain Reaction (PCR) screening of candidate ALD6-EcACS1 containing strains. Lanes 1-5 show PCR products specific to the exogenous *E. coli* ACS1 gene for 5 candidate strains. Lanes 6-7 are positive control PCRs corresponding to the FAS1 gene.

Conclusion

- By employing techniques endemic to genetic engineering, we successfully made an adh1::G418 knockout strain, which was verified through PCR.
- In addition, we overexpressed the ALD6-EcACS1 cassette in an adh1::G418 background.
- Our results indicate that combination of these genetic modifications create an *S. cerevisiae* strain that overexpresses acetyl-CoA.

Future Directions

- As yeast innately lacks the ability to produce fatty alcohols, we aim to express an exogenous mFAR plasmid in the ALD6-EcACS1/adh1 strain, resulting in the conversion from fatty acyl-coA molecules to fatty alcohols [figure 1].
- We intend to compare lipid profiles between the mutant acetyl co-A overexpression strain with mFAR to the wild type *S. cerevisiae* with mFAR, and analyze levels of fatty alcohols produced.
- Ultimately, we aim to optimize the yield of fatty alcohol synthesis for a more environmentally friendly means of production.

Acknowledgements/Works Cited

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[1] Fitzherbert, M.J., *et al.* 2008. How will oil palm explansion affect biodiversity? *Trends in Ecology & Evolution*. **23**: 538-545.

[2] Steen, E.J., *et al.* 2010. Microbial production of fatty-acid-derived fuels and chemical from plant biomass. *Nature*. **463:** 559-562.