Using Frying Oil to Produce High Value Products in an engineered strain of *Escherichia coli*

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INTRODUCTION

Motivation

Imagine this: you're a small restaurant owner with some fryers in the kitchen. Every two weeks, you pay someone to come by and empty out a big barrel of the used frying oil from that week; it's a little bit cheaper since they're going to make it into biofuels, but it still costs a lot and you don't get any return. What if instead of that scenario, every couple of weeks you sell your spent oil for a profit. The company who buys it will use *E. coli* that is genetically modified to break down frying oil and use it to produce a terpenoid, and you avoid the high cost of disposal. This is what our team is working towards.

Background information

Approximately 3 billion gallons per year of used frying oil are produced in the U.S. alone. While some recycling efforts are put forth to turn used frying oil into biodiesel and some companies are working on more effective ways to recycle the frying oil, the iGEM team at Colorado State University is working to turn spent frying oil into a high value product. There are four major components to this year's project including; the breakdown of frying oil, a biosensor for detecting the breakdown of frying oil, production of a high value product, and a kill switch to kill the bacteria if they were to be released into the environment. Our team aims to put each of these components into *Escherichia coli.*

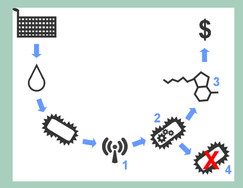

Figure 1. An overview of Using Frying Oil to Produce High Value Products in an engineered strain of *Escherichia coli.* Used frying oil and the cells are combined. 1. Represents the biosensor. 2. Represents breakdown. 3. Represents High-Value Product. 4. Represents Kill switch.

Abstract

The purpose of this project is to create a value added product from used frying oil as the feedstock. *Escherichia coli* is being engineered to break down lipids to acetyl-CoA and produce a high value terpenoid based product through the mevalonate pathway. A biosensor will be incorporated to detect the presence of lipids via the interaction of acyl-CoA with FadR which results in the de-repression of Green Fluorescent Protein. For added security, a kill switch is being developed to destroy the cell if released into the environment. The protein KillerRed is repressed in the presence of tryptophan. In the absence of tryptophan and in the presence of light, KillerRed will kill the cell through the production of reactive oxygen species.

BIOSENSOR

Goals

- Detect the presence of fatty acids
- Function as the promoter for breakdown
- No unnecessary stress without oils present

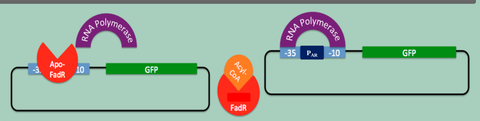

Figure 2. On the left Apo-FadR is pictured bound to the ribosomal binding site inhibiting RNA polymerase from transcribing the gene. On the right Acyl-CoA is bound to Apo-FadR causing it to dissociate from the FadR gene allowing for transcription.

Background

A promoter that functions as a biosensor will be constructed in order to help regulate the breakdown of fatty acids so that there is no stress on the cell if frying oil were not present.

Experimental Methods

- Assemble modified FadR promoter from four sequenced oligonucleotides
- Construct four individual plasmids containing these promoters
- Insert Green Fluorescent Protein (GFP) into these plasmids in order to test functionality

Future Work

Once functionality is determined, choose best promoter to use as a regulatory component of the breakdown process

BREAKDOWN

Goal

- Breakdown used frying oil products
- Create metabolic intermediate acetyl-CoA for high value product manufacturing

Figure 3. Ideal pathway for used frying oil breakdown to high value product

Background

In order to break down the frying oil, we have taken advantage of the cell's natural ability to break down fatty acids for use in the Kreb's cycle. We have up-regulated the limiting enzyme (FadD) that aids in the breakdown of fatty acids in order to have the cell produce more acetyl-CoA.

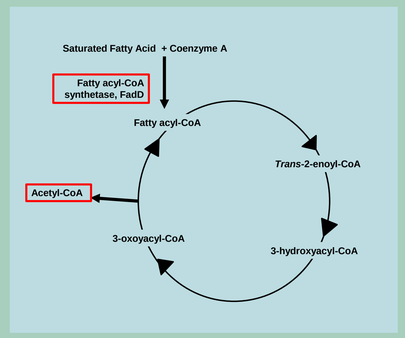

Figure 4. β-oxidation. Boxed is acetyl-CoA, the target breakdown product as well as the FadD enzyme.

Experimental Methods

- Design plasmid for up-regulation of FadD
- Test by linking to Lac promoter

Future Work

- Link to biosensor as a promoter for FadD regulation

HIGH VALUE PRODUCT

Goal

Acetyl-CoA⟶ IPP⟶ Terpenoid

Background

Acetyl-CoA can be used in the mevalonate pathway. This pathway is common to most plants as well as yeast. Genes from yeast will be taken to construct two operons in order to produce isopentenyl pyrophosphate (IPP). While both IPP and dimethylallyl pyrophosphate (DMAPP) are both naturally produced in *E. coli* through the non-mevalonate pathway, the mevalonate pathway provides a more efficient route from acetyl-CoA to terpenoid production than the alternative. By use of the mevalonate pathway any regulatory components that may come with the non-mevalonate pathway can be avoided. Our high-value product will be a terpenoid that has yet to be determined.

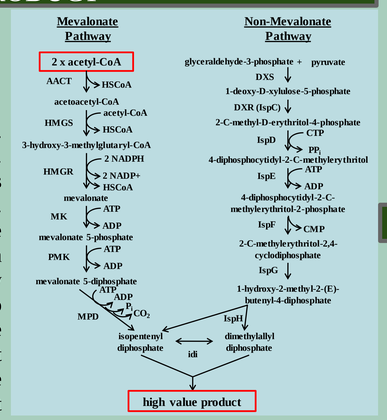

Figure 5. Mevalonate and Non-Mevalonate Pathways

Experimental Approach

•Created plasmids to put mevalonate pathway into *E. coli*

•Based on constructing two operons from yeast genes (*S. cerevisiae)*

Figure 6. Plasmid maps for the two operons constructed for the Mevalonate pathway

Future Work

•Design and create plasmid for terpenoid/ high-value product production

KILL SWITCH

Goal

- Toggleable low-leak kill switch
  - Repressed in the presence of tryptophan
  - Low stress for the cell when repressed
  - Highly-efficient at killing the cell if cell escapes into environment

Background

For our kill switch, a gene named Killer Red which is a mutant of hydrozoan chromoprotein anm2CP from Anthomedusae sp. DC-2005 has been codon optimized for use in plasmid construction. The gene has been inserted into a plasmid construct to provide a fail-safe mechanism in the unlikely event of genetically modified *E. coli* escaping into the environment. The Killer Red gene has a repressible promoter in which tryptophan acts as the repressor. There will be tryptophan present while the cell is used to break down the frying oil, but is not highly present in the environment. Without the presence of tryptophan the Killer Red gene will be activated and white light will produce reactive oxygen species within the cell, causing cell death.

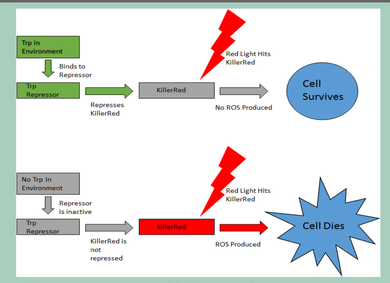

Figure 7. Design of kill Switch. Picture on top there is tryptophan in the environment which binds to the repressor to repress KillerRed so when red light hits the cell no reactive oxygen species (ROS) is produced and the cell survives. Picture on bottom shows low levels of tryptophan in environment so that KillerRed is not repressed and when Red Light Hits the cell ROS is produced and the cell dies.

Experimental Approach

1) Construct two plasmids
- Trp + GFP Plasmid
- Trp+ KR Plasmid

2) Kill curve of KR plasmid

Future Work

- Model efficiency of Trp promoter and kill curve for KillerRed

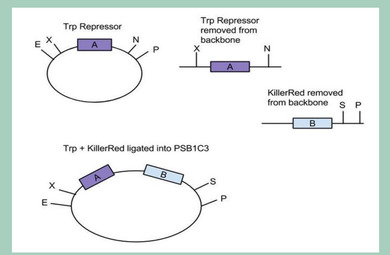

Figure 8. Plasmid construct of KillerRed with tryptophan repressor.

ACKNOWLEDGEMENTS AND REFRENCES

References

Bulina, M., et. al. "A genetically encoded photosensitizer." Nature Biotechnology. 95-99. 2005. October 2014.

Dobarganes, M. Carmen. "Formation of New Compounds during Frying - General Observations." AOCS Lipid Library. Web. 2 February 2009. June 2014.

Kameda, Kensuke, et al. "Purification and Characterization of Acyl Coenzyme A Synthase from Escherichia coli." The Journal of Biological Chemistry, 11. Web. 10 June 1981. June 2014.

Martin, Vincent J., Douglas J. Pitera, Sydnor T. Withers, Jack D. Newman, and Jay D. Keasling. "Engineering a Mevalonate Pathway in Escherichia Coli for Production of Terpenoids." *Nature Biotechnology* 21.7 (2003): 796-802. *PubMed.gov*. Web. 27 June 2014.

Zhang, Fuzhong, James M. Carothers, and Jay D. Keasling. "Design of a Dynamic Sensor-regulator System for Production of Chemicals and Fuels Derived from Fatty Acids." *Nature Biotechnology* 30.4 (2012): 354-59. Web.

Zhang, Hanxing, et al. "Molecular effect of FadD on the regulation and metabolism of fatty acid in Escherichia coli." FEMS Microbiology Letters, 2. Web. 16 May 2006. June 2014.

Colorado State University's iGEM team would like to thank our advisers Dr. Christie Peebles and Dr. Ashok Prasad as well as Jiayi Sun, our graduate student adviser, for all of their support and dedication to helping our team members complete this years project. We would also like to thank the Lucas Argueso lab at Colorado State University for their donation of genomic yeast DNA. And all of our sponsors who helped make this years project possible.