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

Adriana Collings1, Krista Henderson1, Chauncy Hinshaw1, Renee Plomondon1, Josiah Racchini1, Savannah Roemer1, Anthony Roulier1, Matt Sabel1, Olivia Smith1, Dr. Christie Peebles2, Dr. Ashok Prasad2

1'2014 iGEM Team Member, *Faculty Advisor*

**INTRODUCTION**

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

**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.

**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

**BREAKDOWN**

**Goal**
- Tollgate 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 KillerRed which is a mutant of hydronium chromoprotein amn2CP from Anthomedusae sp. DC-2005 has been codd optimized for use in plasmid construction. The gene has been inserted into a plasmid construct to provide a safe-fail 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 is the repressor.

**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

**KILL SWITCH**

**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.

**Experimental Approach**
- Created plasmids to put mevalonate pathway into *E. coli*
- Based on constructing two operons from yeast genes (*S. cerevisiae*)

**Future Work**
- Design and create plasmid for terpenoid/high value product production

**ACKNOWLEDGEMENTS AND REFERENCES**

**BIOSENSOR**

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

**Background**
A biosensor 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 FadD 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

**REFERENCES**

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