Using frying oil to produce trans-zeatin in an engineered strain of *E. coli*
Background

3 billion gallons produced per year in US[1]

Breakdown used frying oil to produce zeatin

2. Photograph courtesy of Dan Dickinson. Used with permission under creative commons with attribution.
Our Project

Extracellular Breakdown → Transport → Intracellular Breakdown → MEP Pathway → Zeatin
Why frying oil?

High energy

Cheap

\[
\text{Fatty acids} \quad \{ \quad \text{Glycerol} \quad \}
\]
Limiting Steps in Breakdown

Breakdown of Frying Oil Components

Transport of Fatty Acids

Breakdown of Fatty Acids
Breakdown of DAGs

Lipase from *Bacillus stearothermophilus*

Turns DAGs into FAs and Glycerol

http://www.emhmagazine.com/uploaded/images/123035292.jpg
Transport of LCFAs into Cell

Natural Transporter: long-chain fatty acid outer membrane porin

Encoded for by fadL
Increased Breakdown of FAs

Beta-oxidation pathway

*fadD* encodes limiting enzyme
fadD Experiment

![Graph showing E. coli Growth on 0.04% Steric Acid]

**E. coli Growth on 0.04% Steric Acid**

- **Graph Description:**
  - The graph illustrates the growth of E. coli over time when cultured with 0.04% Steric Acid.
  - The x-axis represents time in hours, ranging from 0 to 12.
  - The y-axis represents OD600, a measure of the optical density at 600 nm, which is an indicator of cell density.
  - Two lines are plotted:
    - Blue line labeled "fadD" indicates a significant growth pattern.
    - Red line labeled "lac Promoter" shows a different growth pattern compared to the fadD line.
  - Error bars are present, indicating variability in the measurements.
Growth Experiment with Used Frying Oil
Growth Experiment with Used Frying Oil

**E. coli Growth on 100% Used Frying Oil**

![Graph showing cell mass over time for E. coli growth on 100% used frying oil.]

**Growth after 4 days on 50% Frying Oil**

![Bar chart comparing cell counts for different conditions: lac promoter, fadL-50%, fadD+fadL-50%.]
Growth Experiment with Used Frying Oil

Initial 50% Solution

50% Solution after 12 hours of Growth
Future Improvements

Quantitative vs. Qualitative Data

Cell Growth Measurement

Optimize Feed and Growth Conditions

Metabolic Engineering
Trans-zeatin

Very expensive

Currently only used in research

Many other applications

Plant Growth Experiment

Biological Activity of the tzs Gene of Nopaline Agrobacterium tumefaciens GV3101 in Plant Regeneration and Genetic Transformation (Zhao-Fen Han et al., 2013)
MEP Pathway

D-Glyceraldehyde 3-phosphate → MEP → Pyruvate

Hexamethylene diamine tetramethylene phosphonic acid (HMBDP)

http://www.pet.hw.ac.uk/research/fast1/research/res_images/chem_inhibitors/hmbdp.gif
Zeatin Biosynthesis

HMBDP

Trans-zeatin Synthase

zeatin riboside 5'-phosphate  \rightarrow  N6-dimethylallyladenine  \rightarrow  zeatin

LOG

hydroxylation

Hexamethyleneamine trimethylenephosphonic acid (HMBPA)

http://www.pet.hw.ac.uk/research/land/landresearch/plants/hibrida/hmdp.gif

Zeatin Construct Growth Experiment

![Graph showing strain growth over time with two lines: one for trans-zeatin and another for lac Promoter Control.]
Solid Phase Extraction Methods

1. Column Wash
2. Bind Supernatant
3. Wash
4. Elute
Zeatin HPLC Results

A) Bioreactor Control

B) Bioreactor Trans-zeatin Strain

C) Trans-zeatin Retention Standard

D) Shake Flask Control

E) Shake Flask Trans-zeatin Strain

F) Trans-zeatin Retention Standard
Design Considerations

Bulina, M., et. al. "A genetically encoded photosensitizer."
Kill Switch

- No IPTG:
  - No KillerRed expression
  - Exposed to light
  - Cell survives

- IPTG feed:
  - KillerRed expression
  - Exposed to light
  - Cell dies
KillerRed Improvement

- Functional improvement
  - Removed trp promoter
- Characterization improvement
  - Collected data on efficacy
Kill Switch Constructs

Improved characterization of KillerRed biobrick

Suspected mutation

Working in a jury-rigged darkroom with KillerRed
Interlab Study

![Graph showing GFP fluorescence intensity for different constructs and biological replicates. The x-axis represents different constructs and controls, while the y-axis represents fluorescence intensity (arbitrary units). The graph includes data for biological replicates 1, 2, and 3. The data suggests variability in fluorescence intensity across different constructs and controls.]
Spoke with experts and compiled articles on:

- Laws and regulation of GMOs
- Commercialization and industry
- Intellectual property

Bridging the Gap
Key Achievements

• Construction and submission of multiple BioBrick Parts (Bba_KI702000-Bba_K1702005)
• Experimental validation of fatty acid breakdown (BBa_K1702001, Bba_K1702002)
• Addressed questions beyond the bench about IP, commercialization, and bridging the gap between academia, industry, and the public
• Used beyond the bench IP research to file provisional patent
• Improved function and characterization of previously existing BioBrick Part, KillerRed (Bba_K1495000)
• Functional prototype of *E. coli* growing on frying oil
Key Challenges

• Reworking breakdown construct ideas after *fadD* experiment
• Inconclusive results about trans-zeatin production
• Proper handling and experimental design for testing KillerRed
Thank You

Dr. Christie Peebles, Dr. Tom Santangelo, and Dr. Ashok Prasad
Lac Promoter

The lac Operon and its Control Elements

- **lac**
- **CAP binding site**
- **lacZ**
- **lacY**
- **lacA**
- **genes**
- **DNA**
- **message RNA**

**CAP protein**

- **RNA polymerase**

**Repressor protein**

-low glucose**

- Lactose available

-high glucose**

- Lactose unavailable

-low glucose**

- Lactose unavailable

-high glucose**

- Lactose available

"Lac operon-2010-21-01" by Lac_operon.png: G3proderivative work: Tereseik (talk) - Lac_operon.png. Licensed under CC BY 2.0 via Commons - https://commons.wikimedia.org/wiki/File:Lac_operon-2010-21-01.png#/media/File:Lac_operon-2010-21-01.png
Growth Experiment with 100% Used Frying Oil

Before Inoculation

After 4 hours of growth
Fatty Acid Growth Experiment

E. coli Growth on 0.04% Used Frying oil

- fadD + fadL
- fadD
- fadL
- lac Promoter
Why Zeatin?

Benefits:
• Increase lateral bud growth
• Increases immunity against pathogenic attack on plants[1]
• Anti-aging properties
• Green (natural) product
• Synthetic production is complex and low yield

Competitors:
Benzyl adenine
• Synthetic
• Not as well researched
• Zeatin is 10x more effective[2]

1. Grubkinsky, Dominik et al. 'Cis- and trans- zeatin differentially modulate plant immunity'. Plant Signal Behav. 2013 8(7); e24798
Zeatin Biosynthesis
MEP Full Pathway
Trans-zeatin Bioreactor Data
### Killer Red Data

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