Genetically engineering bacteria to degrade and metabolize PET plastics

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INTRODUCTION

Research Background

- **Polyethylene Terephthalate (PET)**
  - Common polymer **used to make various plastic products** (ranging from water bottles to synthetic fibers)
  - Most PET products end up being **dumped into landfills**
  - **Harmful build-up of PET in global ecosystems** due to PET’s inertness and durability
- ***Ideonella Sakaiensis***
  - Species of bacteria discovered in 2016
  - **Degrades PET** into constitutive monomers and **metabolizes** them:
    - **Ethylene glycol (EG)**
    - **Terephthalic acid (TPA)**
  - Problems:
    - **Slow PET degradation** too impractical for waste clean-up
    - **Difficult to scale** due to lack of characterization of the species
  - Solution:
    - Use a **more genetically tractable and characterized bacteria** (i.e. *Escherichia coli*)
    - Use **directed evolution** to improve PET degradation

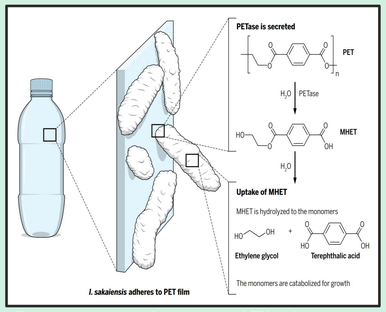

Figure 1. *Ideonella sakaiensis* degrades PET into its constitutive monomers, EG and TPA, using two enzymes, **PETase** and **MHETase** (Bornscheuer, 2016)

Project Objectives

1. Engineer *E. coli* to degrade PET by expressing **PETase** & **MHETase**
2. Engineer *E. coli* to metabolize **ethylene glycol (EG)**
3. Engineer *E. coli* to metabolize **terephthalic acid (TPA)**

END GOAL: Genetically engineer ***Escherichia coli*** to **degrade PET** and **metabolize** the breakdown products **EG & TPA** as **sole carbon sources**

OBJECTIVE #1: Engineer *E. coli* to degrade PET by expressing **PETase** & **MHETase**

Methods

Synthesized the PETase gene, five pelB secretion tag variants, and the necessary amplification primers

↓

Used Gibson Assembly to combine the T7 vector backbone, PETase gene, and each of the pelB secretion tag variants

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Transfected the Gibson Assembly products into an *E. coli* ECNR1 strain with the T7 polymerase with via electroporation

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Grew bacterial colonies and screened for the clones with the desired plasmids using Sanger DNA sequencing

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Induced PETase expression in transfected cells and performed SDS-PAGE to verify that PETase is being secreted

Results

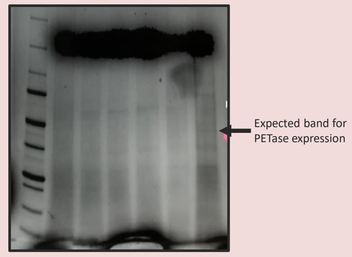

Figure 2. SDS-PAGE gel of various culture supernatants
Lane 1: Ladder
Lane 2: *E. coli* without PETase plasmid
Lane 3-6: ATc-Induced *E. coli* with PETase plasmid

- All 5 PETase-pelB fusions were successfully assembled and transfected into *E. coli*
- SDS-PAGE analysis showed that some PETase may have been secreted, but the results are inconclusive and do not show a significant amount of protein expression
- Cause for the lack of PETase expression is unknown and must be investigated further in future experiments
- Troubleshooting strategies include using a different promoter (pLTetO) as well as super-folded GFP (sfGFP) for improved PETase visualization

OBJECTIVE #2: Engineer *E. coli* to metabolize **ethylene glycol (EG)**

Methods

- **Genomic Integration of Inducible Promoters**
  - Inducible **tetO** and **lacO** promoters were used to **dynamically increase expression** of **fucO** and **aldA**, native genes required for EG metabolism
  - **Lambda red recombination** was used to delete the native promoters and integrate inducible promoters
- **Flux Balance Analysis (FBA)**
  - FBA was used to **identify important pathways** that could be up-regulated to increase EG metabolism
  - The **COBRA Toolbox** for **MATLAB** was used to simulate the metabolism of the starting *E. coli* strain in the presence of increased glycolate, a downstream metabolite of EG metabolism from fucO and aldA
- **Multiplex Automatable Genetic Engineering (MAGE)**
  - Single-stranded **MAGE oligo libraries** were designed to optimize RBS’s of the genes identified from FBA via directed evolution
  - The starting *E. coli* strain was then electroporated with the MAGE oligo library and recovered in a minimal media with EG as a sole carbon source

Results

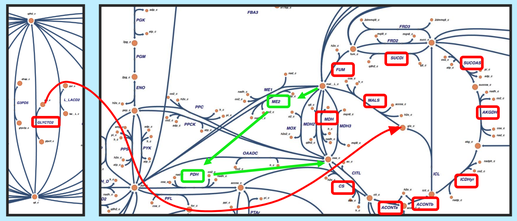

Figure 3. Metabolic flux diagram resulting from FBA of the starting *E. coli* strain’s metabolism in the presence of increased glycolate. Certain genes (boxed in red) were predicted to be expressed at levels ≥2 orders of magnitude higher than basal expression levels. The TCA cycle represents the collective target of this data. FBA was also used to identify genes (boxed in green) which had moderately increased expression but are still required for succinyl CoA, supplementary to the TCA cycle.

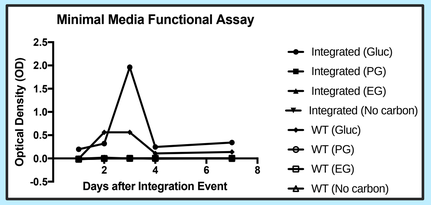

Figure 4. After the new promoters were genomically integrated into the starting *E. coli* strain, the resulting bacteria were grown in various minimal media solutions containing one of 4 sole carbon source conditions: glucose, EG, PG, or no carbon source. Only the bacteria grown in glucose exhibited considerable growth, indicating a need for further genetic engineering and deeper analysis of EG metabolism optimization.

OBJECTIVE #3: Engineer *E. coli* to metabolize **terephthalic acid (TPA)**

Methods

- **TPA Metabolism**
  - To genetically engineer *E. coli* to metabolize TPA, **two foreign gene clusters** are required to first convert TPA into **protocatechuic acid (PCA)** and then convert PCA into succinyl-CoA, which then feeds into the TCA cycle
  - To ultimately achieve TPA metabolism, the *E. coli* first needed to be engineered to metabolize PCA as a sole carbon source
- **Expressing the PCA Degradation Gene Cluster in *E. coli***
  - The PCA degradation gene cluster used was a set of 9 genes native to ***Acinetobacter baylyi* ADP1** that was PCR amplified and cloned into various vectors
  - To test different promoters, the genes were cloned behind an ATc-inducible **T7 promoter** as well as an Atc-inducible **PLtetO-1 promoter** for expression in *E. coli*
- **Adaptively Evolving *A. baylyi* ADP1 to Better Metabolize PCA**
  - To improve *A. baylyi*’s native ability to grow off PCA as a sole carbon source, cultures of *A. baylyi* were **adaptively evolved** by continuous growth and passaging in a minimal media solution containing PCA as the sole carbon source
  - Afterwards, the adaptively evolved bacteria were then exposed to the mutagen **ethyl methanesulfonate (EMS)** and further adaptively evolved in minimal media

Results

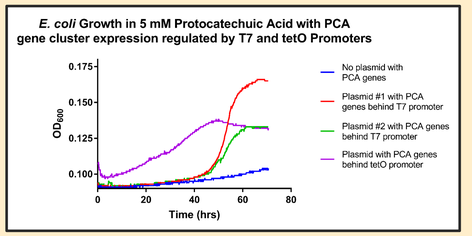

Figure 5. The PCA degradation gene cluster was expressed in *E. coli* using two constructed plasmids with a T7 promoter and one constructed plasmid with a PLtetO-1 promoter. It is not yet clear whether one promoter has an overall advantage over the other. The resulting differences in *E. coli* growth between the two constructed T7 plasmids will be investigated further.

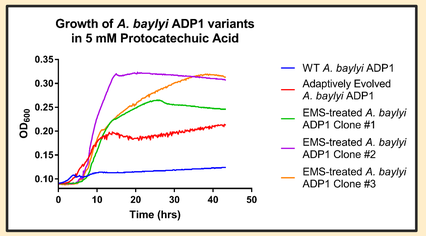

Figure 6. Adaptively evolved *A. baylyi* not only grew faster but also to a higher maximum carrying capacity in 5mM PCA. Additional adaptive evolution after exposure to the mutagen EMS improved growth in 5mM PCA further. The mutations and differentially expressed genes between the variants will be investigated further.

CONCLUSION

Future Work

- **OBJECTIVE #1:** Engineer *E. coli* to degrade PET by expressing **PETase** & **MHETase**
  - Troubleshoot PETase and MHETase expression and secretion in *E. coli*
  - Verify PETase and MHETase activity with Liquid Chromatography-Mass Spectrometry
- **OBJECTIVE #2:** Engineer *E. coli* to metabolize **ethylene glycol (EG)**
  - Optimize *E. coli* growth in EG using Multiplex Automated Genomic Engineering (MAGE) and Flux Balance Analysis (FBA)
- **OBJECTIVE #3:** Engineer *E. coli* to metabolize **terephthalic acid (TPA)**
  - Optimize *E. coli* growth in PCA using long-term adaptive evolution
  - Express TPA metabolism genes to allow for *E. coli* growth in TPA as a sole carbon source

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