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 Solsirensis
  - Species of bacteria discovered in 2016
  - Decomposes 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

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

Methods

- Synthesized the PETase gene, five pEP secretion tag variants, and the necessary amplification primers
- Used Gibson Assembly to combine the T7 vector backbone, PETase gene, and each of the pEP secretion tag variants
- Transfected the Gibson Assembly products into an E. coli ECR1 strain with the T7 polymerase with via electroporation
- Grew bacterial colonies and screened for the clones with the desired plasmids using Sanger DNA sequencing
- Induced PETase expression in transfected cells and performed SDS-PAGE to verify that PETase is being secreted

Results

- All 5 PETase-pEPb 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 (i.e., T7), as well as super-folded GFP (sGFP) for improved PETase visualization

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

Methods

- Genomic Integration of Inducible Promoters
  - Inducible tetr and iso promoters were used to dynamically increase expression of fucO and aldA, native genes required for EG metabolism
  - Lambda red recombinase 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 Automated Genetic Engineering (MAGE)
  - Single-stranded MAGE oligo libraries were designed to optimize RBSs 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

- MinMin Media Functional Assay

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 succinic-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 expression vectors
    - Tests of different promoters, the genes were cloned behind an A. baylyi ADP1 regulated T7 promoter as well as an A. baylyi PL8 promoter-1 promoter for expression in E. coli
  - Adaptively evolve A. baylyi ADP1 to better metabolize PCA
    - To improve A. baylyi’s native ability to grow on TPA as a sole carbon source, cultures of A. baylyi were adaptively evolved by continuous passage and growing in a minimal media solution containing PCA as the sole carbon source
    - Afterwards, the adaptively evolved bacteria were then exposed to the mutant ethyl methanesulfonate (EMS) and further adaptively evolved in minimal media

Results

- Growth of A. baylyi ADP1 variants in 5mM Terephthalic Acid