Biofuels and Bacterial Animations

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Abstract: We aimed to develop a system capable of converting recalcitrant biopolymers into substrates for biofuel synthesis. From pond water, we isolated bacteria capable of metabolizing lignin and polystyrene. We attempted to identify the degradation genes and express them in Escherichia coli. In parallel, we worked to improve ethanol production in E. coli by diverting electron flow from normal cell metabolism to alcohol fermentation. We also explored using Zymomonas mobilis, a more efficient ethanol producer, as an expression host for our biodegradation enzymes. We also aimed to improve the spatial and temporal control of bacterial behavior. We modified the coliroid system to produce a degradable output, allowing a bacterial image to change over time. With this animated coliroid, we worked to create an interface between digital animation and biology using a simple light projector.

Introduction

Our project was divided into three tracks: degrading materials such as plastics and cellulose; increasing biofuel production by using proteorhodopsin pumps and Nuo/Ndh knockouts; as well as other cell types such as Zymomonas mobilis, and collaborating with the California Institute of the Arts to produce animations using fluorescent bacteria. In order to find a degradation pathway, we conducted “gene fishing” experiments on samples taken from several ponds on the Caltech campus to isolate organisms that are capable of degrading biopolymers, and transformed Z. mobilis so that it is able to ferment on substrates outside of what it naturally consumes. In order to improve biofuel production, we incorporated a proteorhodopsin-dependent energy producing mechanism into the cells. Proteorhodopsin is a light-activated proton pump originally found in marine organisms. For the bacterial animation project, we created a construct of a fluorescent protein (mCherry), with a degradation tag attached into a light sensing system. We aimed to develop a system capable of converting incalcitrant biopolymers into substrates for biofuel synthesis. From pond water, we isolated bacteria capable of metabolizing lignin and polystyrene. We attempted to identify the degradation genes and express them in Escherichia coli. In parallel, we worked to improve ethanol production in E. coli by diverting electron flow from normal cell metabolism to alcohol fermentation. We also explored using Zymomonas mobilis, a more efficient ethanol producer, as an expression host for our biodegradation enzymes. We also aimed to improve the spatial and temporal control of bacterial behavior. We modified the coliroid system to produce a degradable output, allowing a bacterial image to change over time. With this animated coliroid, we worked to create an interface between digital animation and biology using a simple light projector.

Degradation: Gene Fishing

We conducted “gene fishing” experiments on samples taken from several ponds on the Caltech campus to isolate organisms that are capable of degrading our substrates, according to the procedure in the above figure. The liquid minimal media cultures were plated on LB after two weeks (see below), and significant growth was observed, indicating that growth had taken place in the minimal media cultures. To further isolate these organisms, each culture was then plated on solid minimal media with the carbon source added, and growth was again observed. As a control, the cultures were also plated on solid minimal media without the carbon source and failed to grow, indicating that they are not using agar as a carbon source. We were able to successfully transfer the pMQ97 plasmids using Gibson assembly to join the necessary components of the pMQ97 plasmid into Z. mobilis.

Human Practices: Bacterial Animations

We constructed BioBricks of mCherry with one of two degradation tags (LVA and AAV) attached, and in parallel inserted mCherry into R0040. We measured the fluorescence of R0040, untagged mCherry, and mCherry with each degradation tag. We also collaborated with students at the California Institute of the Arts and had an exhibit at CalArts on bacterial plate art in which students were able to draw on and make their own plate art.

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