ORGANOFOAM
Genetically Engineering Fungal Mycelium for Biomaterials Development

ABSTRACT

The goal of Organofoam is to develop a toolkit of genetic parts for engineering complex fungi, particularly plant-pathogenic basidiomycetes. Our project was inspired by Ecovative Design, a New York-based start-up company that uses lignin-degrading fungi and plant matter to produce a biodegradable Styrofoam substitute known as "mushroom packaging". Polystyrene, in addition to being tremendously inefficient to recycle, takes hundreds of years to degrade and produces dozens of chemical toxins upon combustion. Ecovative's product has the potential to solve these problems, but their production efficiency suffers from frequent pathogenic mold contamination, a problem that we hope to solve using synthetic biology. Cornell iGEM is developing a fungal genetic toolkit to expand the accessibility of fungal genetic engineering and render it compatible with commercial and industry standards.

BACKGROUND

Economic Assessment

- Increasing prices of polystyrene products in response to rising costs and regulations
- Decreased consumer demand for polystyrene
- Higher demand for environmentally sustainable packaging
- Projected decrease in price difference translates to increased potential economic viability of Organofoam

ENVIRONMENTAL ASSESSMENT

Styrofoam

- 14 million tons produced/year
- Accumulates in landfills and oceans and damages the ecosystem
- Produces toxins upon combustion
- Insufficient and costly to recycle (low rate of recovery, expensive to sort and transport, high temperatures and pressures required)
- Banned from 200+ cities

Chassis

Cochliobolus heterostrophus (BBa_K1021007) — A complex basidiomycete we plan to engineer in the future
- Genetically tractable readily undergoes homologous recombination and, thus, is ideal for gene insertion and deletion at specific sites
- An ascomycete relatively simple to culture in the lab
- Displays a minimal hyphal physiology that carries the material qualities of Ecovate’s product

BIOSAFETY

Our two greatest concerns with commercialization of the genetically engineered product were horizontal gene transfer and loss of biodiversity. To lessen these concerns, we developed preventative safety mechanisms. When implemented, our kill switch system would allow us to quickly initiate cell death. The kill switch system would greatly decrease the chance that our fungal strain could outcompete other organisms and decrease biodiversity. Our kill switch is composed of holm, which degrades the cell membrane, and a chitinase (BBa_K1021006) that is able to degrade the chitinous cell wall.

Fungal Transformation

Fungal transformation relies on the integration of the construct into the genome of the target cell. For unclear reasons, many fungi are unable to incorporate foreign DNA via random insertion in the genome. However, this process has extremely low efficiency relative to homologous recombination, a process that is ubiquitous among all organisms.

We transformed Cochliobolus heterostrophus with our engineered constructs via random insertion and discovered that the inefficacies of the process made it nearly impossible to isolate successful transformations. We have since transformed Cochliobolus using a vector called PING that contains a multiple cloning site flanked by regions from the Cochliobolus genome. With this vector, we have been able to transform BBa_K1021001 into Cochliobolus and demonstrate its ability to confer genetic resistance.

In the future, we would like to efficiently transform Ganoderma Lucidum as well; however, because it is not as well-characterized as Cochliobolus, vectors containing homologous sequences are not readily available. To overcome this limitation, we isolated DNA from two strains of Ganoderma and amplified the 3’ and 5’ regions of glyceraldehyde-3-phosphate dehydrogenase (gpd), which we submitted to the Registry (BBa_K1021011, BBa_K1021012, BBa_K1021013, BBa_K1021014).

TRANSGENIC FSTK UNFITNESS

- Pathogenic mold contamination leads to decreased production efficiency
- Fungal contaminants compete with the growing mycelium during “standoff”
- Secretion of enzymes designed to halt growth of competitor
- Potentially harmful contaminants: Aspergillus fumigatus, Aspergillus niger, and Aspergillus blasticus
- Goal is to express antifungals in mycelium to help it compete with Aspergillus
- Antifungal proteins from Streptomyces tend to specifically target Aspergillus

To efficiently characterize our promoter library, we assembled five constructs with varying promoters, to selectively and strongly express fungal genes under the control of the T7 promoter. This has been an effective tool in engineering, bacterial and mammalian cells, and we hope to demonstrate its use in fungi for the first time.

ANTIFUNGALS

- Gal genetic engineering and render it compatible with commercial and industry standards.
- Synthetic biology. Cornell iGEM is developing a fungal genetic toolkit to expand the accessibility of fungal genetic engineering and render it compatible with commercial and industry standards.

RESEARCH ELEMENTS

We have introduced three new antibiotic resistance genes (BBa_K1021001, BBa_K1021002, and BBa_K1021003) to the Paris Registry. These genes confer resistance to antibiotics, hygromycin, and blasticidin, respectively. We isolated these from those fungal transformation vectors and performed three rounds of site-directed mutagenesis to remove illegal cut sites via site-directed mutagenesis. These parts have also been assembled into composite parts to be expressed under the control of constitutive fungal and viral promoters.

We tested the functionality of the composite part BBa_K1021033 — the hygromycin resistance gene under the control of the T7 promoter—in E. coli BL21-AL using a zone-of-inhibition assay. As shown in the figure to the right, the construct was able to confer a highly significant (p<0.005) level of hygromycin resistance to the cells.

We have also been able to demonstrate the functionality of BBa_K1021001, the generic resistance gene, via direct transformation into Cochliobolus heterostrophus.

CHARACTERIZATION

To efficiently characterize our promoter library, we assembled five constructs with green and red fluorescent protein downstream of viral and fungal promoters. While we have been unable to characterize the relative strengths of the fungal promoters yet, we have demonstrated that the transcriptional regulation of the T7 promoter allows for high levels of expression in BL21 when a bacterial RBS is present.

T7+GFP+RBS and T7+GFP constructs in E. coli BL21 were examined for their relative fluorescence (fluorescence/OD600). A T7 construct in E. coli BL21 without downstream elements was utilized as a control along with the use of the aforementioned construct in E. coli DH5α. As indicated by the graph, there were evident differences in peak fluorescence between the T7+GFP+RBS construct, T7+GFP construct, and controls. The T7+GFP+RBS construct had a 20 fold higher fluorescence than T7+GFP or the controls.

ACCOMPLISHMENTS

- Submitted a comprehensive toolkit of 30 novel BioBricks to standardize fungal genetic engineering
- Demonstrated functionality of components, implemented transformation in complex fungi
- Bridged the gap between synthetic biology and the consumer market through corporate partnership
- Developed a feedback-controlled incubator to optimize fungal growth

SPONSORS

Tim Abbott, Hanik Arai, Ryan Absher, Nipin Bhat, Arun Chakravarty, Jenkin Chen, Rebecca Chen, Shafali Dong, Sara Gregg, Alex Han, Eric Holness, Danielle Huang, Daniel Latch, Rafael Larramendi, Ole Langsethund, Jeffrey Ly, Ritvik Sarfet, Mie Sniert, Paulkherm, Mark Simpson, Christine Song, Olja Spasojevic, Tina Su, Smita Suresh, Yoshiko Takeya, Lydia Wang, Jeff Wheeler

ADVISORS

Drs. B. Gallant Tangren, Xueling Shen, Shrinivas Arlekar, Bruce Land