Dead Lignum Tell No Tales: Development of Synthetic Yeast for Enzymatic Pretreatment of Lignocellulosic Biomass


Purdue University, West Lafayette

Background
- Need to find more sources of renewable energy which can alleviate problems in areas such as food production and water purification
- Ethanol biofuels are promising, but there are drawbacks of using food crops & fertilizers
- Lignocellulosic biofuels avoid food and other resource commitments, but conversion is inefficient due to recalcitrance of lignin to degradation and monomers poisoning crucial enzymes.
- Traditional approaches to circumvent lignin require pretreating with high heat/energy or toxic/expensive chemicals
- Recently, a synthetic yeast reported to be capable of spatially separating the final biofuel conversion steps prevents the poisoning
- An opportunity arises for an enzymatic pretreatment to degrade lignin to increase conversion efficiency

Methodology
1. Identify lignin degrading enzymes from termites and fungi
2. Insert genes into yeast for secretion
3. Add feedstock to pretreatment with recombinant yeast
4. Degraded lignocellulosic biomass moves to biofuel conversion to produce ethanol

Results
- The parts contained overlapping restriction sites
- Successfully Gibson assembled 4 of 6 enzymes
- Oxygen killswitch was completed

Future Directions
- Bradford assay to measure protein expression levels
- Peroxidase assay to measure activity of Lignin Peroxidase, Versatile Peroxidase, and Manganese Peroxidase
- DMP assay to measure laccase activity
- Klason Procedure to determine levels of lignin degradation

Human Practices
- Toured Cardinal Ethanol Plant (Union City, IN)
- Challenges facing cellulosic ethanol production:
  - Economic
  - Infrastructure
  - Implementation
- Outreach:
  - Synbio training for Girl Scouts
  - Mentoring MASI high school students
- Host 4-H workshop for synbio, teach high school students lab technique

Implementation
1. Identify lignin degrading enzymes from termites and fungi
2. Insert genes into yeast for secretion
3. Add feedstock to pretreatment with recombinant yeast
4. Degraded lignocellulosic biomass moves to biofuel conversion to produce ethanol

Conclusions
- Our synthetic biology solution to biofuel conversion from ligno-cellulosic biomass to ethanol did not reach its full potential. Overlapping restriction site errors and gBlock design errors blocked its progress. Better gene design would be necessary for future research. However, we still believe the idea is viable and shows potential for future progress, if supplied more time and resources.
- This project could have extensive applications if a low total cost for our yeast construct was achieved. An ideal standardized platform for bioreactors could be created in the future to allow for consistent testing.

References: