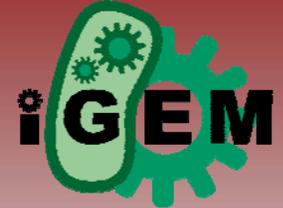




Genetically Engineered System for Lignin Biodegradation

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Introduction

Cellulose, Hemicellulose, and Lignin are the three main components of plant biomass. In fact, Cellulose and Lignin are the most abundant biopolymers on the planet, and they offer an enormous amount of chemical resources. Lignin proves to be the biggest limitation on biomass degradation. Also, its resistance to degradation decreases the availability of the other plant tissue components. Lignin is found in many plant products, including newspaper print, and it lends the 'hardness' to wood materials. Its limitation on wood product bioavailability is due to the complex polymeric structure which proves difficult to break. Therefore, it presents a formidable physical boundary to utilization of biomass resources.

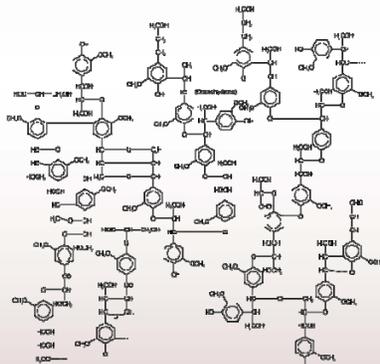
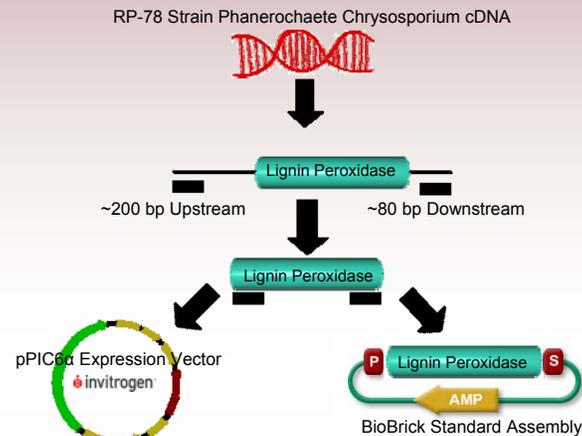


Figure 1: Lignin structure, showing the structural complexity of the polymer.

Goals

- Isolate the enzymes necessary for Lignin biodegradation
- Contribute standardized biodegradation parts to the Registry
- Make it easier to engineer a biodegradation system
- Experiment with degrading other materials that resist degradation like plastics, and other synthetic polymers

Strategy



Methods and Materials

- cDNA obtained from Phanerochaete Chrysosporium grown on Aspen Wood at University of Wisconsin Forest Products Laboratory
- Lignin Peroxidase A (LipA) isolated by Polymerase Chain Reaction using two different sets of DNA primers (Invitrogen)
- LipA cloned into pGEM (Promega) plasmid and grown in XLI-Blue E. Coli
- LipA digested from pGEM with EcoRI and NotI Restriction Enzymes (Promega), cloned into pPIC6α A (Invitrogen), and grown in E. Coli
- pPIC6α A containing LipA transformed into Pichia Pastoris for testing

Results

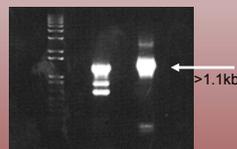


Figure 2: Initial PCR isolation of LipA from BKM(1) and RP-78(2).

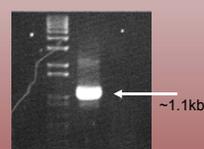


Figure 3: Second PCR isolation of LipA with coding region specific primers.

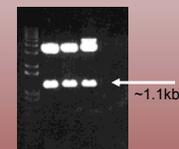


Figure 4: Digestion of pPIC6α containing LipA showing gene insert.

Our Part

Lignin Peroxidase

- Registry Part BBa_144000
- 1117 bp Lignin Peroxidase Isozyme
- Currently under testing and characterization
- Undergoes redox reaction with Veratryl Alcohol and Hydrogen Peroxide substrates
- Used to degrade Lignin and possibly other complex polymers

Accomplishments

- Isolated Lignin Peroxidase from highly homologous gene family
- Cloned gene into yeast expression vector for testing
- Standardized our gene and submitted biodegradation part to Registry
- Helped make it easier to engineer a biodegradation system

Future Work

- Further test and fully characterize our part
- Develop genetic system around our part for lignin degradation
- Isolate and standardize other genes involved in lignin degradation
- Test our enzyme's ability to degrade synthetic polymers

Acknowledgments

We would like to thank Jill Gaskell at the University of Wisconsin and USDA Forest Products Laboratory for her help working with Lignin Peroxidase, and the cDNA from Phanerochaete Chrysosporium.

