Towards Renewable Petrochemicals

Engineering biology to convert waste into fuel

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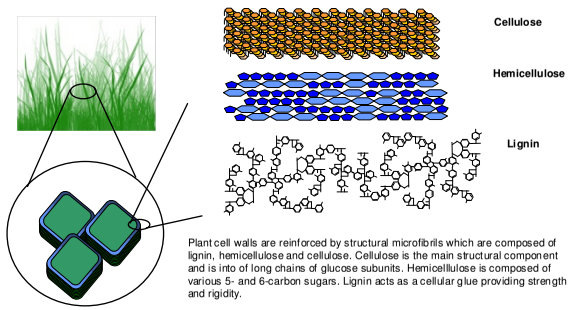
Synthetic Biology Standards Network

EPSRC Engineering and Physical Sciences Research Council

Introduction

One of the major goals in the field of synthetic biology is the biosynthesis of commodity chemicals and fuels from renewable sources. Plant biomass is touted as the most attractive carbon source for this application due to its high abundance and cheap cultivation. Cellulose is the major biopolymer of plant biomass, however is resistant to microbial degradation due to a crystalline structure and intimate association with other carbohydrate polymers.



Plant cell walls are reinforced by structural microfibrils which are composed of lignin, hemicellulose and cellulose. Cellulose is the main structural component and is into of long chains of glucose subunits. Hemicellulose is composed of various 5- and 6-carbon sugars. Lignin acts as a cellular glue providing strength and rigidity.

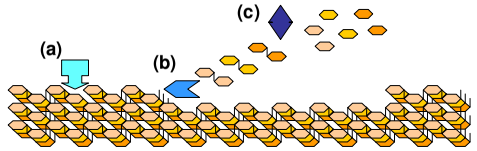
Considerable research has focused on the development of a consolidated bioprocessing microorganism, capable of simultaneous cellulose saccharification and product synthesis. Yet the recalcitrant nature of plant biomass to microbial degradation remains a major hurdle in this effort. In the current research we present a 'parts-based' approach to this problem and leverage several concepts and tools within synthetic biology.

Research objectives & strategy

- To construct a library of defined BioBrick parts, each encoding an enzymatic function for the breakdown of cellulose or hemicellulose.
- To explore synergistic effects between individual cellulases.
- To generate 'plug and play' genetic devices encoding predefined activities against specific cellulosic substrates.
- To contribute towards the development of a microbial production host capable of utilising plant biomass as a renewable feedstock.

Non-complexed cellulase systems

Complete hydrolysis of cellulose in non-complexed cellulase systems entails the concerted action of three enzyme sub-families; endoglucanases, exoglucanases and β-glucosidases.

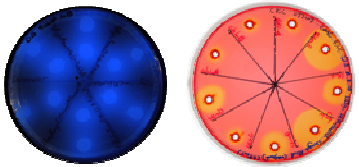


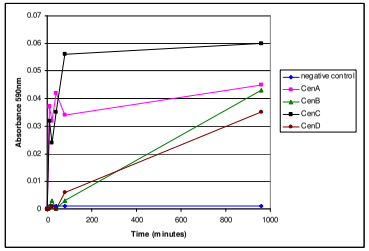
Endoglucanases are shown to cleave at internal sites along the cellulose polymer **(a).** Exoglucanases processively move along the chain cleaving cellobiose residues **(b),** which are in turn hydrolysed by β-glucosidases to glucose subunits **(c).**

Parts characterization

A BioBrick parts library encoding cellulolytic and hemicellulolytic activities is sourced from the soil bacterium *Cellulomonas fimi.* Construction and characterization of each part is performed in *Escherichia coli (JM109).*

Fluorescently tagged substrates such a methylumbelliferyl-cellobioside exhibit a a β-1,4 bond which when cleaved will release the fluorogenic methylumbelliferyl molecule. This test identifies the action of exoglucanases **(a).** Amorphous cellulose dyed with Congo red dye will produce zones of clearing in the presence of endoglucanases **(b).**





Time course analysis of four endoglucanases from *C. fimi* active against carboxymethyl-cellulose.

Carboxymethyl-cellulose dyed with Remazolbrilliant Blue dye acts as a substrate for testing the activites of four endoglucanases. The rate at which dyed sugar residues are liberated from the cellulose polymer is measured as a function of absorbance at 590nm.

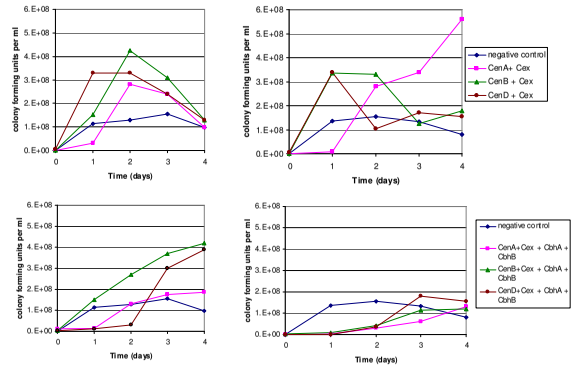
Results indicate the endoglucanases hydrolyse amorphous cellulose at varying rates.

Results

Using a rational design approach, composite parts are assembled, transformed and expressed in a novel expression host. The growth rates in minimal media containing cellulose as a sole carbon source are measured. Preliminary results identify strains capable of cellulose utilisation.

Experiment 1: Strains utilising crystalline cellulose

Experiment 2: Strains utilising amorphous cellulose



Experiment 1 suggests that multiple exoglucanases are required for efficient utilisation of crystalline cellulose. Whereas growth in *Experiment 2* on amorphous cellulose is less demanding, requiring only the expression of a single endoglucanase and single exoglucanase.

Conclusions

- Proper characterization of a standardised library of BioBrick parts can aid in the rational design of devices encoding predefined activities against specific cellulosic substrates.
- Recombinant expression hosts require multiple cellulases to utilise cellulose as a carbon source. The composition of this enzyme cocktail is largely dictated by the target substrate.
- Preliminary results highlight the potential in applying a 'parts-based' approach to the design of a bioprocessing host and the power of standardising biological engineering.