142. The Unexpected Importance of Actinobacterial GH12 in Hydrolysis of Crystalline Cellulose

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Project Goals: The primary research goal of the JBEI Deconstruction Division is to discover and optimize methods by which biomass can be efficiently converted into fermentable sugars and aromatics. The specific goal of this project is to discover and optimize lignocellulolytic enzymes and enzyme mixtures for enhanced hydrolysis of pretreated biomass.

Production of fermentable sugars via enzymatic hydrolysis of complex plant polysaccharides is a subject of intense global interest. Here, we investigated compost enrichments as sources of novel thermophilic enzymatic mixtures, referred to as cocktails, for cellulose hydrolysis. Bacterial consortia were adapted to microcrystalline cellulose (MCC) at 60°C under aerobic conditions using Berkeley Green Waste (BGW) compost as the inoculum. 3,5-dinitrosalysilic acid (DNS) and p-nitrophenol (pNP) enzyme assays indicated enrichment supernatants had high levels of glycoside hydrolase activities. Isolated supernatants were then used to successfully saccharify MCC and 1-ethyl-3-methylimidazolium acetate ([C2C1Im][OAc]) pretreated switchgrass (ILSG). Together, these data demonstrate that BGW consortia are an excellent source of enzymes for development of enzymatic cocktails. In addition, supernatants were tolerant of ionic liquids, emerging chemicals for biomass pretreatment, indicating the enzyme in these supernatants may be useful in industrial-scale biofuel production. Microbial community compositional analysis revealed that Actinobacteria dominated early passages of two enrichment lineages. One lineage sustained Actinobacterial dominance over two-week intervals, while the community profile of a second lineage changed from predominantly Actinobacteria to a mixture of Bacteroidetes and Firmicutes. These data suggest a succession in primary and secondary cellulose degraders in these cultures that may be important in understanding the mechanism of cellulose hydrolysis by thermophilic bacterial populations. The supernatant from the Actinobacterial-dominated lineage was particularly effective at saccharification of crystalline cellulose. Comparative metagenomic and proteomic analyses revealed differential expression of glycoside hydrolases (GHs) from Actinobacteria were responsible for the changes in observed activities on biomass substrates. In particular, the abundance of GH12 from the Actinobacteria correlated with activity of the supernatants on microcrystalline cellulose. This result was unexpected because GH12 is commonly characterized from bacteria and fungi as an endoglucanase with negligible activity on crystalline substrates. Heterologous expression of the Actinobacterial GH12, along with GH6 and GH48 from the same organism (a close relative of Thermobispora bispora) demonstrated that it had high activity on CMC and pNPC substrates. Heterologous expression of these GHs (GH12, GH48, GH6, AA10) has provided purified enzymes to formulate an enzymatic mixture for high-temperature saccharification of biomass substrates with crystalline cellulose.

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143. Droplet Microfluidics for Synthetic Biology

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http://www.jbei.org/research/divisions/technology/microfluidic-assays/

Project Goals: Synthetic biology experiments require optimization of pathways consisting of many genes and other genetic elements and given the large number of alternatives available for each element, optimization of a pathway can require large number of experiments consuming prohibitively-expensive amounts of DNA and enzymes. Droplet microfluidics, because of its ability to process small volumes, presents a cost-effective solution for conducting high-throughput cloning and expression experiments. Unfortunately, these platforms are generally not designed in a robust nature with the feedback and temperature control necessary for screening large combinatorial libraries. Towards these ends, we are developing devices that integrate droplet microfluidics, digital microfluidics and peltier heaters/coolers. These microfluidic systems will be utilized for automating all critical steps of synthetic biology including DNA assembly, transformation, culture and expression. We will utilize these technologies for screening a variety of biological constructs relevant to the mission statement of the Joint BioEnergy Institute.

Rapid and inexpensive synthesis of plasmid-sized DNA would dramatically accelerate synthetic biology research. We are developing an integrated system for rapid DNA synthesis by iterative construction of single DNA molecules in a microfluidic device (Figure 1A). As reagents are dispensed to the reaction chamber in nanoliter quantities, reaction progress is monitored by single-molecule fluorescence on a custom-built total internal reflection fluorescence (TIRF) microscope. Once a single DNA molecule with the desired sequence is constructed, it is amplified for use in synthetic biology applications.

Synthetic biology has become an approach to understand and to manipulate biological systems in bacteria and in yeast for biofuel applications. However, given this extremely challenging goal, the biological design cycle (specify-design-construct- verify) is currently very slow, expensive, and laborious. To expedite this process, significant efforts are needed to develop enabling technologies for rapid biological engineering (e.g. an automated platform for constructing designs and verifying the constructs in a host organism). A microfluidic device has been developed which allows DNA assembly and verification (Figure 1B). This system was utilized to assemble 16 different plasmids using three different DNA assembly methods: Golden Gate, Gibson, and yeast assembly.

Transformation of exogenous DNA into bacterial cells is a powerful technique for genetics studies. A highly integrated device based on digital microfluidics and strategically positioned peltiers allows all transformation procedures to be carried out on a single device (Figure 1C). Three peltiers were positioned below the device to provide the 4oC, 30oC, 37oC and 42oC temperatures employed during chemical heat shock. The digital microfluidic device allows automation of all critical steps of transformation and culture including plasmid addition, transformation by heat-shock, addition of selection medium, culture and protein expression. Flexibility in device operating parameters allows transformation of various DNA plasmids into many different cell types.

A platform that is a combination of classic-droplet- microfluidics and digital microfluidics is being developed (Figure 1D). The grouping of these two techniques allows us to take advantage of both platforms' strengths, negating their pitfalls. To demonstrate the device's ability, we are conducting a study for the directed evolution of enzyme ionic liquid tolerance. The device will be able to combine reagents, incubate, and screen for enzyme activity at high throughput. Only droplets with enzymes that

can efficiently generate glucose in the presence of ionic liquids will be collected for further mutagenesis of the isolated DNA.

We have developed devices that integrate droplet microfluidics, digital microfluidics and peltier heaters/coolers. These microfluidic systems have been utilized for automating all critical steps of synthetic biology including DNA assembly, transformation, culture and expression. Future plans are to expand the applications of this technology to other projects that are in need of a highly-controlled yet high-throughput platform, such as gene design and protein pathway engineering.

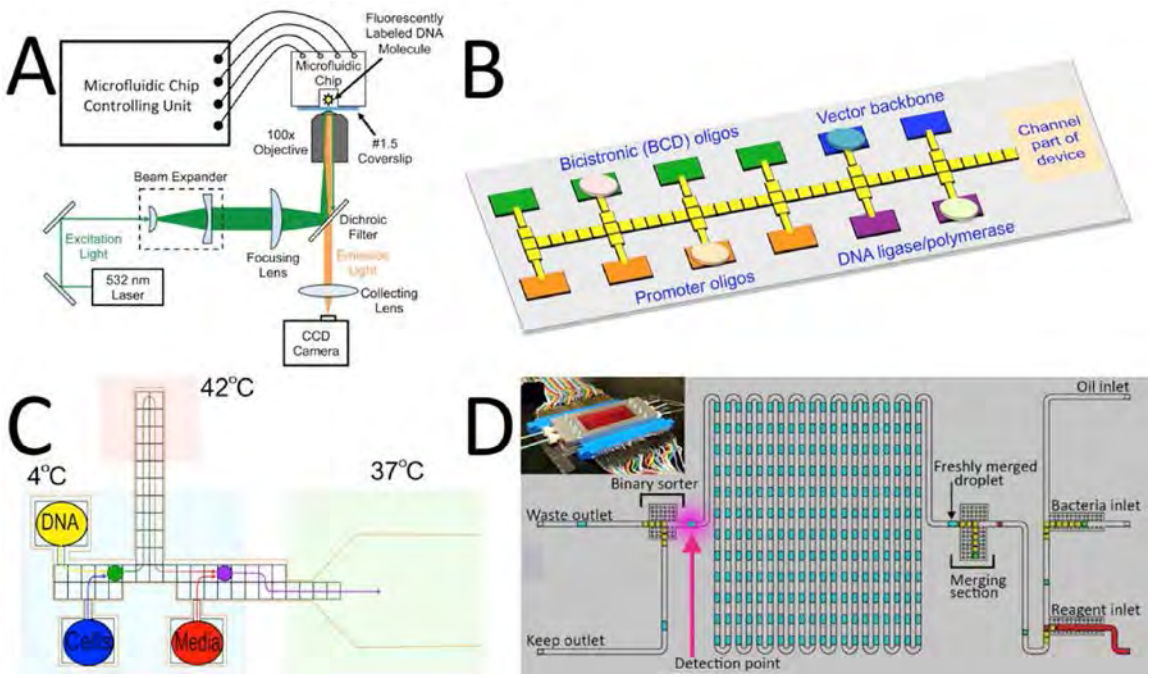

Figure 1. A) Schematic of microfluidic chip based DNA synthesis system on TIRF microscope for fluorescent single molecule detection. B) Schematic of microfluidic device for DNA assembly and verification C) Schematic of a DMF device for cell transformation. D) Photo and schematic of the architecture and function of a chip for directed evolution of ionic liquid tolerance.

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