

132. Universal expression tools to improve nutrient acquisition of energy crops

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<http://www.jbei.org/research/divisions/feedstock/cell-wall-engineering/>

Project Goals: The main goals of this research are the generation of “universal” expression tools for plant root engineering and to utilize them to improve nutrient acquisition in energy crops.

Plant growth and development relies on roots as a means to anchor the plant body as well as to absorb water and nutrients such as N, P and K. As deficiency in these nutrients negatively impacts photosynthesis, plant growth and yield, chemical fertilizers have been used to compensate and meet the growing demand for plant material. Unfortunately the excessive use of fertilizers has come at high environmental and economic costs; and its production utilizes a substantial proportion of worldwide energy consumption. The current development of growing crops on marginal land (low water content, low nutrient supply, vulnerability to erosion and heavy-metal pollution) for bioenergy will reduce competition with food crops and the pressure on high-quality arable lands utilization. As sufficient nutrient uptake by the plant root is vital especially when soil conditions restrict its availability, root systems in energy crops can be engineered to acquire/ accumulate the required nutrients.

Our focus is to design “universal” expression tools for plant root engineering functional across diverse plant species. Through targeted-cell engineering and synthetic biology we aim to engineer plant root systems to generate metabolic pathways and improve nutrient acquisition/accumulation in energy crops. This aim will be carried out through development and validation of a large set of root-specific and nutrient-responsive promoters. In house transcriptome analysis will help identify root specific genes that are constitutively expressed, induced or repressed through conserved metabolic responses to N, P and Fe starvation in hydroponic conditions across a widely diverse selection of plant species. Promoters from the identified genes will be isolated and characterized for spatiotemporal expression patterns and expression levels across different taxonomic classes using composite plants and conventional transgenic approaches. Subsequently monocot and dicot “universal” promoter libraries of the detected genes alongside those previously reported will be generated using a semi-high-throughput promoter cloning approach and yeast homologous recombination assembly method and ultimately used for tissue specific metabolic pathway engineering to improve nitrogen use efficiency in bioenergy crops.

We expect that the results of this research will generate a diversity of building blocks for plant engineering and will directly contribute to advance the DOE’s mission for the sustainable production of bioenergy.

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133. Genetic Parts Screening and Artificial N-glycosylation Motif Engineering for

Heterologous Protein Production in *Aspergillus niger*

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Project Goals

The principal goal of the Fungal Biotechnology Team is building an efficient fungal platform for heterologous gene expression in fungi, which accommodates the array of largely prokaryotic enzymes with superior properties discovered and characterized in the other teams of the Deconstruction Division at JBEI. The main platform organism is *Aspergillus niger* and we are applying systems biology approaches to understanding heterologous enzyme production, and employing that understanding for the engineering of a better protein production platform.

Abstract

Aspergillus niger is a genetically tractable model organism for scientific discovery and a platform organism used in industry for the production of enzymes. Expression of secreted native enzymes at tens of grams per liter have been discussed by those in industry, but high level production of heterologous enzymes remains elusive. Strategies to increase production include the use of strong promoters, protease-deficient strains, fusion proteins, multiple gene copies, etc. However, yields of heterologous proteins are still lower than desired. In collaboration with the Environmental Molecular Sciences Laboratory (EMSL), we generated proteomics data from secretome samples of *A. niger* grown on a variety of minimal and rich media, with the goal of identifying useful genetic elements for increasing heterologous protein production. Twenty promoters, six signal sequences and four introns from the most highly secreted proteins were identified as candidate genetic elements to enhance heterologous gene expression. These candidate elements were tested for their ability to drive expression of a prokaryotic glycoside hydrolase. A vector was designed to target integration of the modified expression cassette to the native glucoamylase gene locus by homologous recombination. Considerable diversity was seen in heterologous protein production driven by these various elements. Interestingly, one promoter for a membrane protein and a non-classical signal peptide showed promising results. In addition, based on the 3D structure of a heterologous glycoside hydrolase, we generated seven individual artificial N-glycosylation motifs on the surface of the heterologous protein and had the genes synthesized in a collaboration with the Joint Genome Institute (JGI). Analysis of these modified proteins for glycosylation, using EMSL's top-down proteomics capability, is underway.

Correlation of glycosylation with any changes in enzyme kinetic and thermodynamic properties of the altered proteins is the goal of this aspect of the research.