



*Using molecular biotechnology to develop novel biofuel feed-stocks with enhanced bio-processing characteristics, improve biomass yield and quality*

Biofuels are liquid and gaseous fuels derived from organic matter, and they have the potential to supplement or eventually replace petroleum-based fuels. Falling fuel supplies have led to increased research and production of biofuels. In 2011, well over 90.2% of current fuel ethanol production capacity in the US is based on corn grain at a level in excess of 6 billion gallons. The primary use of ethanol feedstock such as corn for food, feed, and fuel has created a significant food-fuel controversy.

This situation has pushed the US, which is the world's largest producer of fuel ethanol, to search for various alternative feed-stock to corn, improving the efficiency of ethanol production process, and increasing the productivity of primary ethanol feed-stocks.

Our research goal is to harness the use of both molecular biotechnology and system biology to improve the speed of converting lignocellulosic and starchy feedstocks into biofuels, and other high value fuels and chemicals, with cleaner and more efficient energy-use profiles.

Cassava, a tuber crop, native to Brazil, grows in diverse environments, especially extremely harsh climatic conditions, and its starch is already being used for large-scale ethanol production in many Asian and African countries. Cassava contains 20-40% starch, about 70% moisture, with a theoretical ethanol yield of 0.45 L kg<sup>-1</sup>. Cassava starch costs 15-30% less to produce per acre than corn starch, making cassava an attractive and strategic source of renewable energy.

Through funding from USDA, the broad aims of this research initiative are to metabolically engineer

cassava, a starchy feedstock, to simultaneously express hyperthermophilic starch-hydrolyzing enzymes required for complete starch degradation to single sugars. We anticipate that this would eliminate the need to add microbial enzymes in starch liquefaction and saccharification, reduce the cost of starch hydrolysis, simplify this process, and increase ethanol yields. We will train a new generation of under-represented minority students in advanced bioenergy research, and gene expression technology, and expose them to new careers in agricultural and molecular biotechnology, and bioenergy.

We have recently developed and molecularly characterized a 24kb "mega plasmid" consisting of tuber-specific multi-gene expression cassettes containing the *P. furiosus*  $\alpha$ -amylase (a starch liquefying enzyme) and amylopullulanase (a starch debranching enzyme), *Sulfolobus solfataricus* glucoamylase (a starch saccharifying enzyme), and modified *E. coli* ADP-glucose pyrophosphorylase (GlgC) genes (to enhance starch synthesis and yield) that target their expression simultaneously only to cassava tuberous roots as a way to develop "self-processing or self-digestible" starch derived from the transgenic cassava.

We anticipate that through this research initiative, we will demonstrate the power of transgenic technology to develop "novel" biofuel feedstock with significantly improved bio-processing characteristics and potential commercial application.

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