UNIVERSITY OF FLORIDA

Engineering Biocatalysts for Hemicelluloses Hydrolysis and Fermentation

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Project Description:

Goals and Objectives:
Our goal is to develop biocatalysts for the cost-effective production of fuel alcohols and chemical feedstocks from underutilized sources of renewable biomass and evolving energy crops. To reach this goal protocols for efficient saccharification of hemicellulose fractions from these resources will be developed.
Objectives are to:

1. Develop improved enzyme-mediated saccharification protocols of hemicelluloses with existing bacterial biocatalysts for production of biofuels and chemical feedstocks.
2. Develop Gram positive biocatalysts for direct conversion of hemicelluloses to biobased products.
3. Develop systems with bacterial biocatalysts for efficient bioconversion of the hemicellulose fractions of perennial energy crops (poplar, eucalyptus, switchgrass, energy cane) to targeted products.

Budget: $192,000
Universities: UF
External Collaborators: Collaborations are in various units within the University of Florida: L.O. Ingram and K.T. Shanmugam, Microbiology and Cell Science; F. Altpeter, Agronomy; G. Peter, Forest Resources and Conservation

Progress Summary

1. *Development of a bacterial biocatalyst for the complete conversion of hemicellulose hydrolysates to biobased ptoducts.*

Genes encoding endoxylanases and alpha-glucuronidases have been identified in Gram negative *Xanthomonas* spp. These have been cloned for expression in Gram negative ethanologenic biocatalysts with type 2 secretion systems, including *Klebsiella oxytoca*. Formation of these enzymes in K. oxytoca may provide a biocatalyst for direct conversion of glucuronoxylans, the predominant polysaccharide in the hemicelluloses fractions from hardwoods and agricultural residues, to biofuels and chemicals. This will extend applications of the patent UF #12617, "Biocatalyst for complete conversion of hemicellulose to biobased products" and potential interests for licensing.

2. *Develop improved enzyme-mediated saccharification protocols of hemicelluloses with existing bacterial biocatalysts for production of biofuels and chemical feedstocks*

Endoxylanases, alpha-glucuronidases and arabinofuranosidases, encoded by genes from mesophilic *Paenibacillus* sp. JDR-2 and the extreme thermophile *Thermotoga maritima*, have been produced as recombinant enzymes for the efficient conversion of the xylans of hemicelluloses to ethanol using the biocatalysts *Klebsiella oxytoca* P2 and *Enterobacter asburiae* E1. In collaboration with Fredy Altpeter in Plant Cell and Molecular Biology, genes encoding endoxylanases from *Thermotoga maritima* have been cloned and expressed in tobacco and

sugarcane to produce quantities of enzymes to use as amendments during pretreatment for saccharification and fermentation. These studies have now been published:

Kim, J.Y., K. Musa, W. Fouad, G. Nong, J.F. Preston and F. Altpeter. 2010. Production of hyperthermostable GH10 xylanase Xyl10B from *Thermotoga maritima* in transplastomic plants enables complete hydrolysis of methylglucuronoxylan to fermentable sugars for biofuel production. Plant Mol. Biol. On-Line ahead of print. 2010.

3. **Develop Gram positive biocatalysts for direct conversion of hemicelluloses to biobased products**

Genes encoding intracellular xylanase and alpha-glucuronidase from *Paenibacillus* JDR2 have been engineered to include secretion sequences for *Bacillus* spp. Transformation of *Bacillus subtilis* with these modified genes has provided new strains that efficiently ferment glucuronoxylan to ethanol, lactate and butanediol. Further downstream engineering of these strains for homoethanol and homolactate fermentation will provide biocatalysts more efficient and cost-effective conversion of woody biomass to fuels and chemicals. This will extend applications of the patent UF# 12619, " Xylan-Utilization Regulon for Efficient Bioprocessing of Hemicellulose and Uses Thereof", and potential interests for licensing.

Funds leveraged/new partnerships created

Targeting plant cell wall degrading enzymes to mitigate pathogenesis of *Xanthomonas axonopodis* pv. *citri*
USDA Citrus Canker Funds
J.F. Preston as PI, J. Jones Co-PI 06/01/10-08/30/11 $84,000 Total and Direct
My on-going collaboration with Professor Jeffrey B. Jones, Dept. Plant Pathology, University of Florida, has been concerned with defining proteins, including enzymes, that contribute to virulence of plant-pathogenic bacteria. Genomic sequence comparisons of different *Xanthomonas* spp. have identified gene clusters that express xylanolytic enzymes. These can be used to engineer Gram negative bacterial biocatalysts, e.g. *Escherichia coli*, *Klebsella oxytoca*, and *Enterobacter asburiae* strains to efficiently convert hemicelluloses to targeted products. We are now engineering strains *Klebsiella oxytoca* to secrete enzymes that will allow direct conversion of woody biomass (forest biomass, agricultural residues and energy crops) to fuels and chemicals.

Next-Generation Sweet Sorghums: Sustainable Production of Feedstocks for Fuels, Chemicals and Value-Added Products
USDA
W. Vermerris (PI) et al. (8 Co-PI's) 4 yr $4,800,000 J.F. Preston (20% commitment) $800,000
Previous collaborations with faculty in the Genetics Institute, including Professor Wilfred Vermeris, have led to the production and chacterization of xylanolytic enzymes in plants, with the goal of production of plant biomass for pretreatment and conversion to fuels and chemicals. This newly awarded grant and support from FESC are complementary with respect to the development of bacteria biocatalysts for direct conversion of cellulosic biomass to fuels and chemicals. Funds ($50,000) from our FESC budget, as well as salaries provided by the University of Florida, provided matching funds for this award.