FESC Florida Energy Systems Consortium

Thrust Area 3: Biomass (Biochemical Conversion)

Engineering Biocatalysts for Hemicelluloses Hydrolysis and Fermentation

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Description: 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: $192,000.00
Universities: UF
External Collaborators: NA

Progress Summary

Develop improved enzyme-mediated saccharification protocols of hemicelluloses with existing bacterial biocatalysts for production of biofuels and chemical feedstocks.
A combination of endoxylanases from Gram positive bacteria have been expressed as recombinant enzymes for the efficient depolymerization of glucuronoxylans, the predominant hemicellulose components of hardwood biomass. As secreted enzymes these have been shown to release oligosaccharides for efficient assimilation and complete intracellularconversion to fermentable xylose. Along with an intracellular alpha-glucuronidase these have been identified for engineering bacterial biocatalysts for the efficient conversion of lignocellulosics to bioethanol.

A thermophilic xylanase has been produced as a recombinant protein in tobacco and sugarcane for production of quantities for saccharification of lignicellulosics on an industrial scale.

Develop systems with bacterial biocatalysts for efficient bioconversion of the hemicellulose fractions of perennial energy crops (poplar, eucalyptus, sugarcane, sweet sorghum) to targeted products.
The genome sequence of *Paenibacillus* sp. JDR2 has been completed, and has identified additional genes with which to engineer l bacteria biocatalysts with genes encoding arabinases as well as xylanases. These will be useful for the saccharification of glucuronoarabinoxylans from grasses.

A strain of *Bacillus subtilis* has been engineered with genes from *Paenibacillus* JDR2 for the efficient conversion of glucuronoxylans from poplar and glucuronoarabinoxylans from sugarcane to targeted fermentation products. This genetically pliable species shows particular promise for the development of a biocatalyst for the consolidated bioprocessing of lignocelliulosic biomass from agricultural residues and energy crops to biofuels and chemicals.

Funds leveraged/new partnerships created: A grant entitled "Next-Generation Sweet Sorghums - Sustainable Production of Feedstocks for Fuels, Chemicals and Value-Added Products" has been awarded to the University of Florida for four years starting 05/11/2011. Wilfred Vermerris from the Department of Agronomy is the PI, with seven Co-PI's including myself. This will provide a total of $509,915 direct cost for my efforts to pursue topics related to the development of enzymes for saccharification and bacterial biocatalysts for direct conversion of lignocellulosics from sweet sorghum to fuels and chemicals.

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Different forms of lignocellulosic biomass represent major renewable resources derived from solar energy via photosynthesis. Several of these are abundant in the southeastern United States and amenable to development as major sources of fuels and chemicals. Energy crops, poplar and energy cane, and agricultural residues, sugarcane bagasse and sorghum, are candidates for bioconversion to targeted products. The hemicellulose fraction, representing 20 to 30% of these resources, may be efficiently converted, via secreted xylanolytic enzymes, to sugars for intracellular metabolism and conversion to biofuels and chemicals by fermentative bacterial biocatalysts. We have identified and characterized xylan-utilization systems from bacteria at the gene and enzyme level, and applied the appropriate enzymes for efficient conversion of xylans to fermentable pentoses, xylose and arabinose. This has led to the identification of bacteria for the secretion of xylanolytic enzymes, assimilation of the products of extracellular depolymerization of xylans, followed by efficient intracellular metabolism. Xylanolytic bacteria, e.g. *Paenibacillus* spp., are candidates for downstream engineering to produce lactate or ethanol. Other bacteria capable of fermentation, e.g. *Bacillus subtilis*, have been engineered for secretion of xylanolytic enzymes for optimal conversion of hemicelluloses to lactate and ethanol. These developments may provide new biocatalysts for consolidated bioprocessing of hemicelluloses for cost-effective conversion of lignocellulosic resources to alternative fuels and chemicals.

Publications:

Kim, J.Y., K. Musa, W. Fouad, G. Nong, **J.F. Preston** and F. Altpeter. 2011. 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. In press.

Potnis, N., K. Krasileva, V. Chow, N. F. Almeida, P. B. Patil, R. P. Ryan, M. Sharlach, F. Behlau, J. M. Dow, M.T. Momol, F. F. White, **J. F. Preston**, B. A. Vinatzer, R. Koebnik, J. C. Setubal, D. J. Norman, B. J. Staskawicz, J. B. Jones. 2011. Comparative genomics reveals diversity among xanthomonads infecting tomato and pepper. BMC Genomics 2011, 12:146 http://www.biomedcentral.com/1471-2164/12/146

St John, F. J., J. C. Hurlbert, J. D. Rice, **J. F. Preston** and E. Pozharski. 2011. Ligand Bound Structures of a Glycosyl Hydrolase Family 30 Glucuronoxylan Xylanohydrolase. J. Molec. Biol. 407:92-109.

Patent application filed:

U.S. Provisional Application SN 60/982,623. UF# 12619. Xylan-Utilization Regulon for Efficient Bioprocessing of Hemicellulose and Uses Thereof. Preston, J.F., V. Chow, G. Nong, J.D. Rice, and F.J. St. John. Filed 10/22/2008

U.S. Provisional Application SN 61/115, 722 UF #12617 "Biocatalyst for complete conversion of hemicellulose to biobased products". Preston, J.F., C. Bi, and J.D. Rice. Filed 11/18/2008