UNIVERSITY OF FLORIDA

Engineering Biocatalysts for Hemicelluloses Hydrolysis and Fermentation

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Project 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

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.**

This support allowed Changhao Bi to complete his Ph.D. and contributed to the development of a new strains of *Enterobacter asburiae* JDR-1 that efficiently converted hemicelluloses hydrolysates to either D-lactate or to ethanol. Relevant publications from this effort include:

Bi, C., X. Zhang, J.D. Rice, L.O. Ingram, J.F. Preston. 2009. Genetic engineering of *Enterobacter asburiae* strain JDR-1 for efficient D(-) lactic acid production from hemicellulose hydrolysate. Biotechnol. Lett. 31:1551-1557.
Bi, C., X. Zhang, L.O. Ingram, J.F. Preston. 2009. Genetic engineering of *Enterobacter asburiae* strain JDR-1 for efficient ethanol production from hemicellulose hydrolysates. Appl. Environ. Microbiol. 75:5743-5749.

A relevant patent application from this effort is:

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.

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

Endoxylanases and alpha-glucuronidases encoded by genes from mesophilic *Paenibacillus* sp. JDR-2 and the extreme thermophile *Thermotoga maritima* have been produced as recombinant enzymes in *E. coli* the provide catalysts for the efficient conversion of the xylans of hemicelluloses to ethanol using the biocatalysts *Klebsiella oxytoca* P2 and *Enterobacter asburiae* E1. Additional studies are in progress to refine the conditions to maximize the conversion of hemicelluloses from forest resources and agricultural residues to ethanol as a biofuel and D-lactate as a chemical feedstock for bioplastics.

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

The definition of the xylan-utilization regulon in *Paenibacillus* sp. JDR-2 has been further refined with the recently completed sequence of the genome of this bacterium. This has identified the combination of the transcriptional regulators, transporters and intracellular enzymes that collectively assimilate the products of extracellular depolymerization of xylans and convert these to fermentable xylose. This has provided evidence for a process in which assimilation and of metabolism of the products of depolymerization is coupled to the depolymerization process that is catalyzed by a cell-associated endoxylanase, allowing efficient and rapid conversion of xylans to fermentable xylose by single bacterial biocatalysts. The results of this discovery are the subject of a publication:

Nong. G., J.D. Rice, V. Chow, and J.F. Preston. 2009. Aldouronate utilization in *Paenibacillus* sp. JDR-2: Physiological and enzymatic evidence for coupling of extracellular depolymerization with intracellular metabolism. Appl. Environ. Microbiol. 75:4410-4418.

and also provided supporting information for a provisional patent application:

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.

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