FESC
Florida Energy Systems Consortium

UNIVERSITY OF FLORIDA

Engineering Biocatalysts for Hemicelluloses Hydrolysis and Fermentation

PI: James F. Preston

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

Progress continues in the following areas, as described below.

- Development of a bacterial biocatalyst for the complete conversion of hemicellulose hydrolysates to biobased ptoducts.
- Development of improved enzyme-mediated saccharification protocols of hemicelluloses with existing bacterial biocatalysts for production of biofuels and chemical feedstocks
- Develop Gram positive biocatalysts for direct conversion of hemicelluloses to biobased products

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

The strain *Enterobacter asburiae* E1 has been used to ferment the acid hyrolysates (Bi et al., 2009), including *Eucalyptus grandis*, *Lirodendron tulipifera* (yellow poplar), and *Liquidambar stryiciflua* (sweetgum), all of which are candidate energy crops for Florida and the southeastern United States. The unique ability of *E. asburiae* to ferment methylglucuronoxylose as well as xylose and xylobiose provides a distinct advantage other biocatalysts currently used to ferment acid hydrolysates of hardwoods.

Industrial Interest has been expressed in the licensing of 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. An international patent application has been submitted for this on 6/30/2010.

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

Efficient utilization of lignocellulosic polysaccharides by bacteria requires either a thermochemical release of fermentable sugars or the secretion of glycohydrolases, including beta-1,4 -endoglucanases (cellulases) and beta-1,4-endoxylanases. Additional enzymes including arabionfuranosidases and esterases may also be secreted to process xylans for depolymerization and assimilation. The extracellular depolymerization of the methyglucuronoxylans is catalyzed by endoxylanases in glycohydrolase families GH5 (or GH30), GH10, GH11. Members of each of these families catalyze the cleavage of a xylosidic bond by acid-base catalysis in which a glutamate residue serves as a nucleophile and a second glutamate serves as a proton donor, resulting in a double displacement reaction in which the anomeric β-configuration of the reactant and product is retained. Of these only the GH10 endoxylanases, which catalyze the release of xylobiose, xylotriose, and the aldouronate MeGAX3, generate products all of which may be directly assimilated by xylanolytic bacteria. A diagram depicting the structure of the O-acetyl-arabinomethylglucuronoxylan and the processing provided by the different options is presented below in Fig. 1.

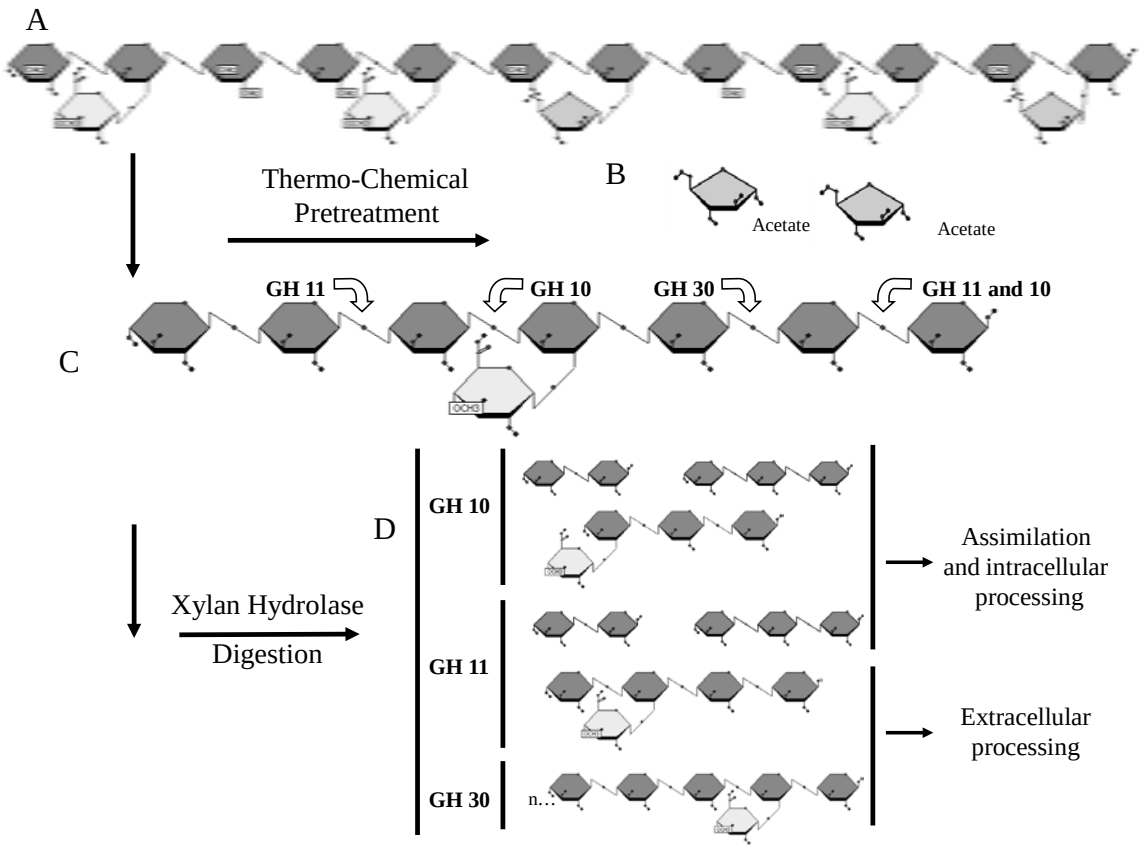


Fig. 1 Pretreatment of O-acetyl-arabinoglucuronoxylan and enzymatic depolynerization of 4-O-methylglucuronoxylan. A) Structural presentation typical for hardwood xylan as a linear chain of β-1,4-lined xylopyranose residues variably substituted at the 2-O positions with α- 4-O-methyl-D-

glucuronpyranosyl residues, and at the 2-O and 3-O positions with α-L-arabinofuranosyl residues or acetyl ester groups. B) Thermochemical pretreatments to release arabinofuranose and acetate from soluble 4-O-methylglucuronoxylan (MeGAXn) C) Digestion of MeGAXn with endoxylanases of glycohydrolase families GH 10, GH11, or GH30, and D) release of aldouronates and xylooligosaccharides for direct assimilation or further extracellular processing.

Endoxylanases, alpha-glucuronidases and arabinofuraonisidases, 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. This fermentation potential has been evaluated further for the efficient digestion MeGAXn and to release fermentable xylose, xylobiose and arabinose. In collaboration with Fredy Altpeter in Plant Cell and Molecular Biology, genes encoding GH10 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. Additional studies are in progress to refine 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*

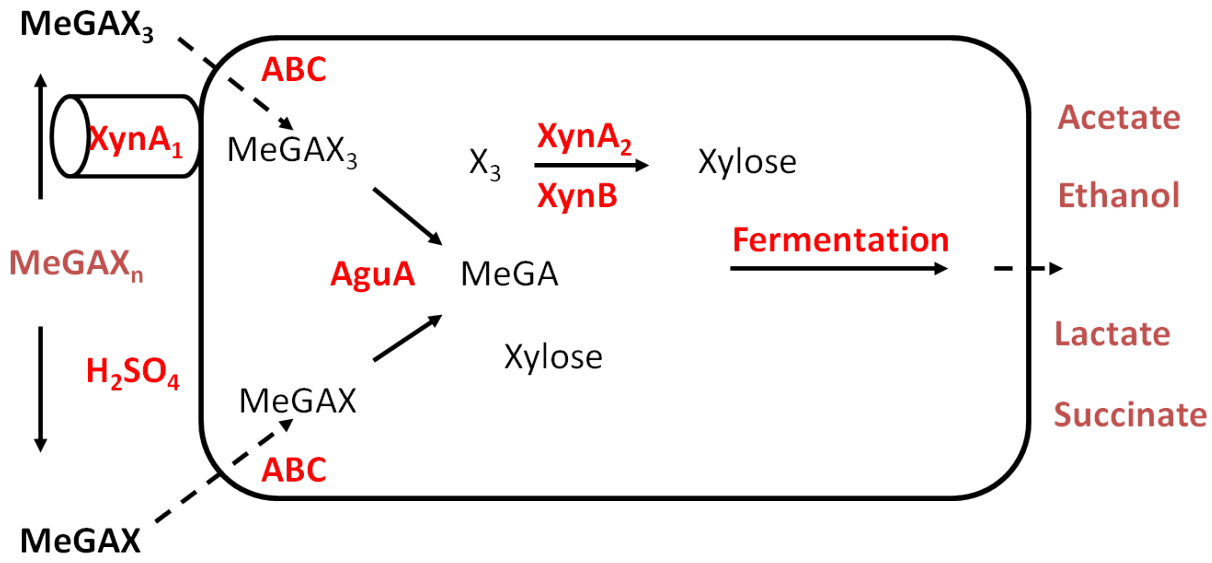
An aggressively xylanolytic bacterium *Paenibacillus* sp. JDR-2, isolated from decaying hardwood, secretes a multimodular GH10 xylanase (XynA1) anchored to the cell surface. Genes encoding transcriptional regulators, ABC transporter proteins, a GH67 α-glucuronidase (AguA), a GH10 xylanase catalytic domain (XynA2), and a putative GH43 β-xylosidase/a-furanosidase (XynB), have been located within a contiguous sequence in the genome of this bacterium. All of these genes as well as that the XynA1 xylanase are coordinately responsive to xylan induction and glucose repression, and collectively comprise part or all of a xylan-utilization regulon. Physiological and enzymatic studies support a process in which the MeGAX3 and xylooligsaccharides released by XynA1 anchored to the cell surface are directly assimilated and metabolized. Growth rates and yields with MeGAXn substrate and the absence of detectable products of repolymerization support a process in which depolymerization and assimilation are coupled.

In the study presented here, the genes that comprise the xylan-utilization regulon in the genome of *Paenibacillus* sp. JDR-2 were used to identify orthologous genes in the genomes of other bacteria. The arrangements as well as sequences of these genes were examined to identify themes to predict a molecular basis for the formation of enzymes that contribute to the coupling of depolymerization to assimilation and the metabolism of the assimilated products. The identification of regulatory genes has also provided insight into the genetic requirements for applying these regulons for the efficient conversion of metylglucuronoxylans to targeted products.

Bioinformatic analysis of sequenced bacterial genomes has identified genes orthologous to those that comprise the xylan-utilization regulon defined in *Paenibacillus* strain sp. JDR-2. Relationships of extracellular depolymerization of methyl-glucuronoxylans, assimilation of the products of depolymerization, and intracellular conversion to fermentable xylose will provide a basis for development of bacterial biocatalysts for direct conversion of hemicellulose to biobased products. Physiological, biochemical and genetic studies with selected bacteria support a process in which cell-anchored multimodular GH10 endo-xylanases catalyze the release of substrates, aldotetrauronate (methylglucurono-xylotriose), xylotriose, and xylobiose, that are directly assimilated and metabolized. The rapid rate of methyl-glucuronoxylan utilization and growth, along with the absence of detectable

products of depolymerization in the medium, indicate that assimilation and depolymerization are coupled processes. Genomic comparisons provide evidence that such systems occur in xylanolytic species in several genera, including *Clostridium*, *Geobacillus*, *Paenibacillus*, and *Thermotoga*. These systems as depicted below may be used, either in their native configurations or through gene transfer to other organisms, to develop biocatalysts for efficient production of fuels and chemicals from the hemicellulose fractions of lignocellulosic resources.

Bacterial Biocatalyst for Direct Conversion Hemicellulosic Glucuronoxylans to Biofuels and Chemicals



An international application has been filed on 06/03/2010 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.