Bacterial Conversion of Hemicellulosic Xylans to Biofuels and Chemicals

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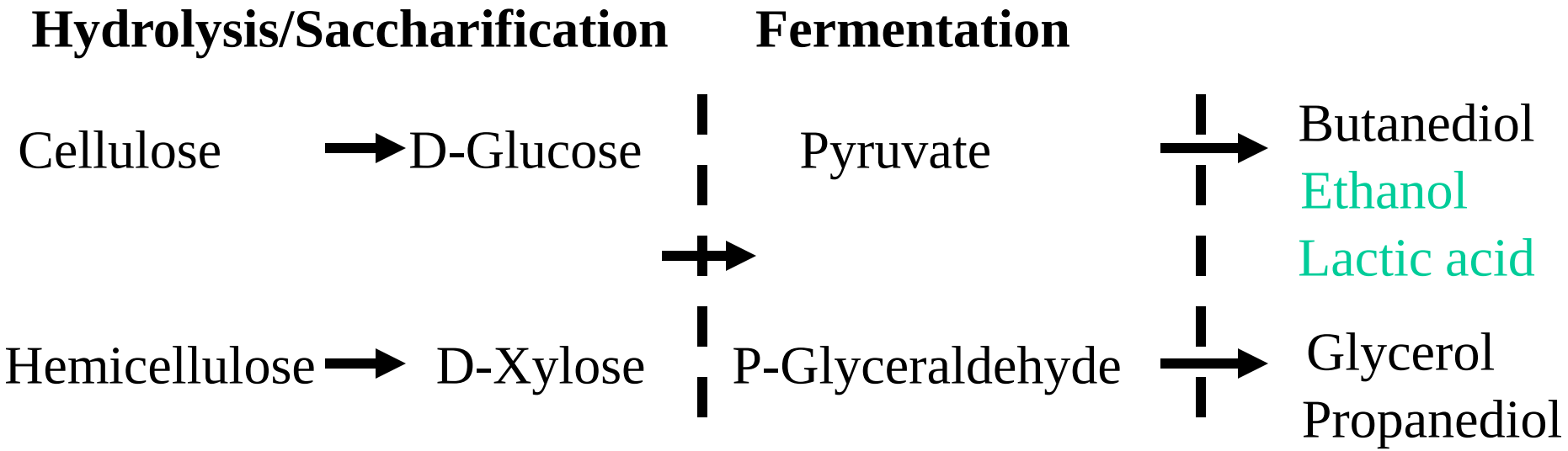
Objective

Genetically engineer bacteria for digestion and fermentation of the hemicellulose fractions of agricultural residues (straw and sugar cane bagasse) and energy crops (poplar, eucalyptus and energy cane) to ethanol and lactic acid

Expected Outcome

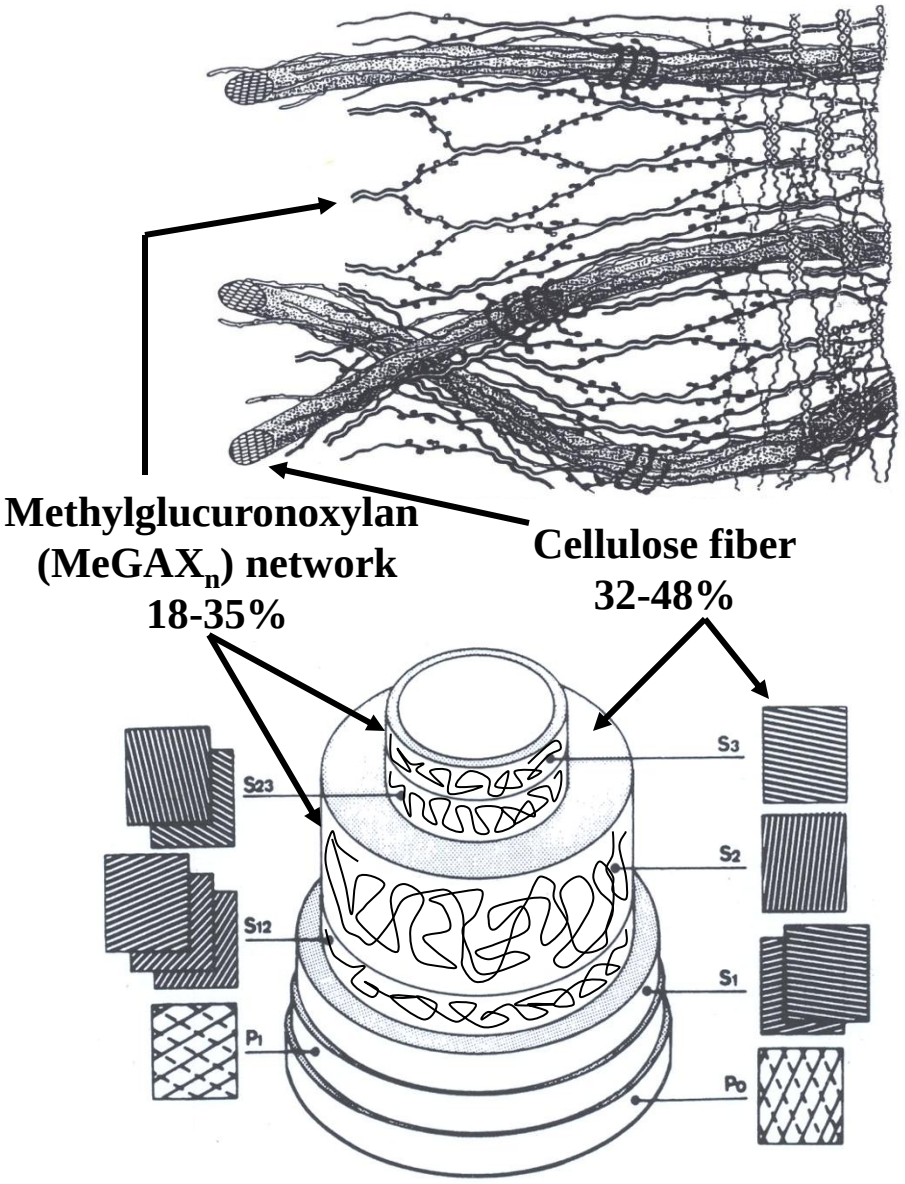
Development of biocatalysts for the cost effective production of fuel alcohols and bioplastics from underutilized renewable resources

OBJECTIVE: Develop biocatalysts to provide maximal yields of alternative fuels and chemicals from lignocellulosics.



SPECIFIC AIM: Develop bacterial biocatalysts that efficiently convert hemicellulose-derived glucuronoxylan to ethanol and chemical feedstocks.

Interaction of Major Polymeric Sugars in Lignocellulosic Biomass



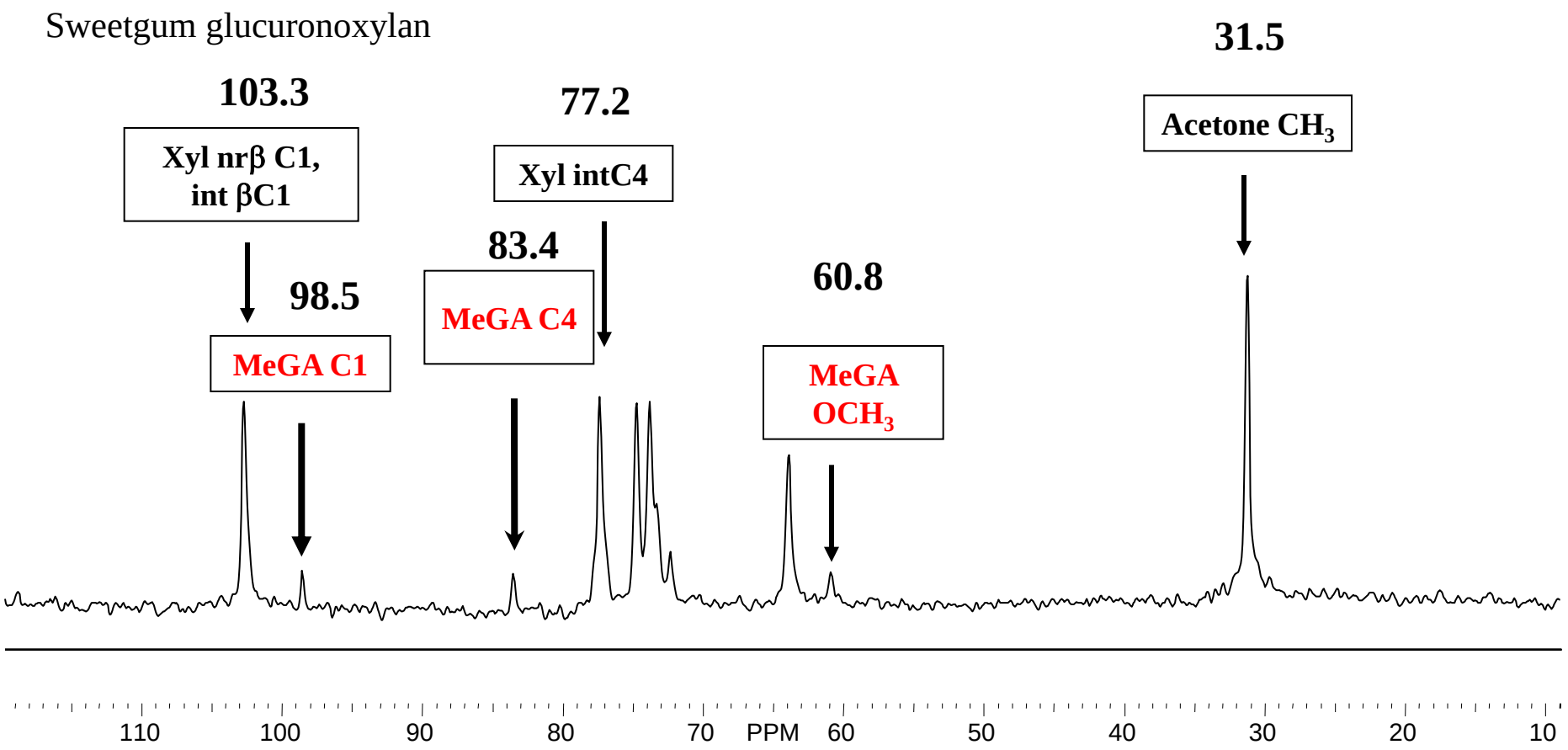
- Cellulose and glucuronoxylan are tightly associated
- Cellulose fibers form through hydrogen bonding interactions between individual cellulose stands
- and cellulose fiber association by coating oGlucuronoxylan acts to limit microfibril r interacting with surface cellulose strands
- The noncarbohydrate polymer lignin embeds the interacting cellulose and glucuronoxylan through ester linkages to glucuronoxylan

Composition of Selected Ligncellulosic Resouces, % dry weight

Feedstock	Glucan (cellulose)	Xylan (hemicellulose)	Lignin
Corn stover	37.5	22.4	17.6
Corn fiber	14.28	16.8	8.4
Pine wood	46.4	8.8	29.4
Popular	49.9	17.4	18.1
Wheat straw	38.2	21.2	23.4
Switch grass	31.0	20.4	17.6
Office paper	68.6	12.4	11.3

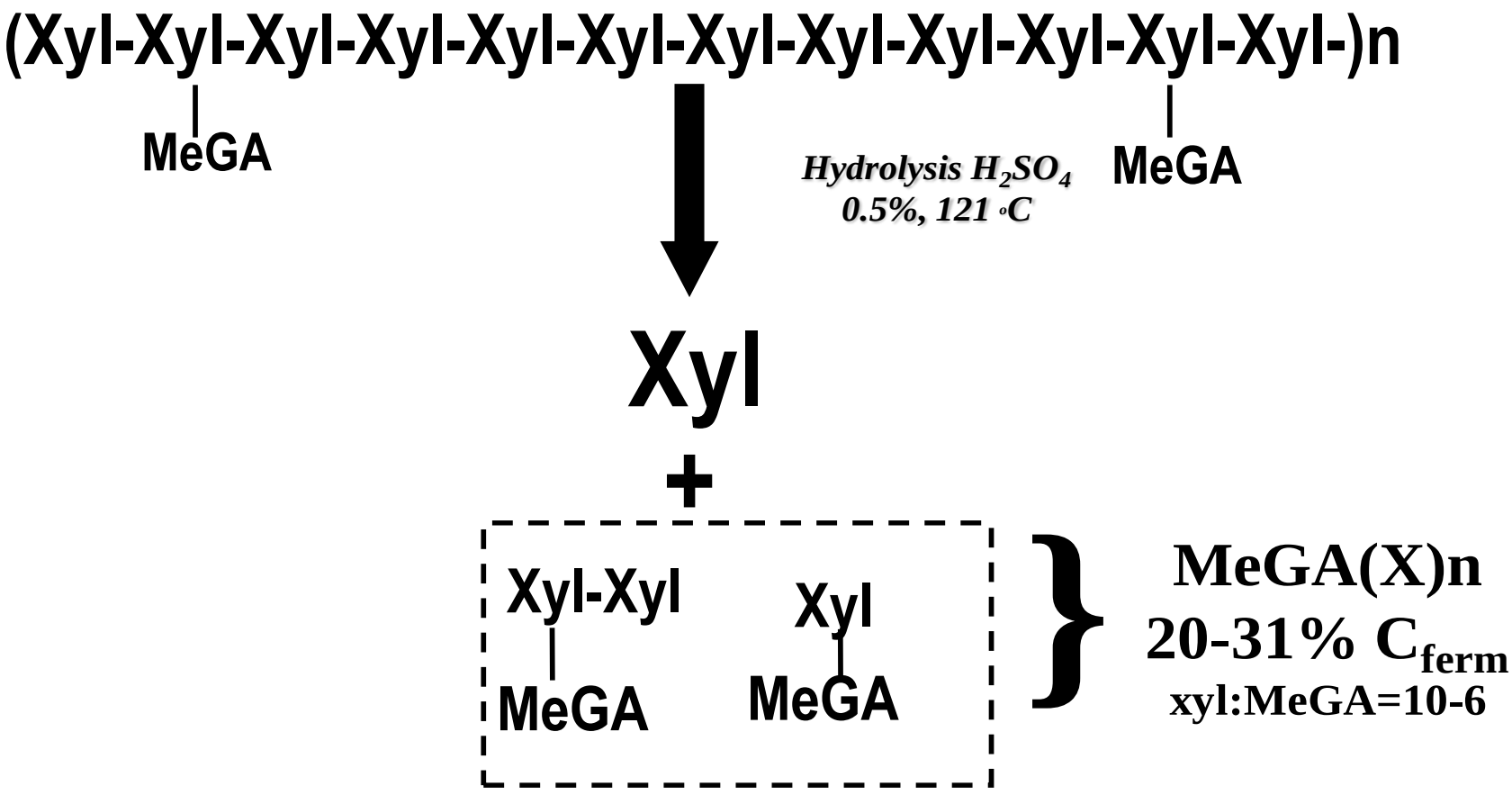
Adapted from N. Mosier et al., 2005. Biores Technol. 96:673-686

Quantification of MeGA substitution 4-O-methylglucuronoxylan by ^{13}C-NMR



Xylose/MeGA	
Sweetgum	6-7
Yellow Poplar	6-7
Sugarcane bagasse	>10

Dilute Acid Hydrolysis of Methylglucuronoxylan



Xylose fermentation products from *E.asburiae* JDR-1 and *E.asburiae* JDR-1 E1 (*pfl*- , pLOI155)

	Fermentation products (mM)						
	Succinate	Lactate	Formate	Acetate	Butanediol	Ethanol	Ethanol yield (% of theoretical)[a]
***E. a.* JDR-1**	**12.7**	**5.6**	**15.0**	**25.2**	**13.4**	**42.6**	**19.2**
***E. a.* JDR-1 (pLOI555)**	**2.2**	**1.2**	**3.6**	**4.2**	**ND**	**217.4**	**98.0**

a: Yield of 100% theoretical defined as 5 mole ethanol formed/ 3 mole xylose consumed.

Summary 1

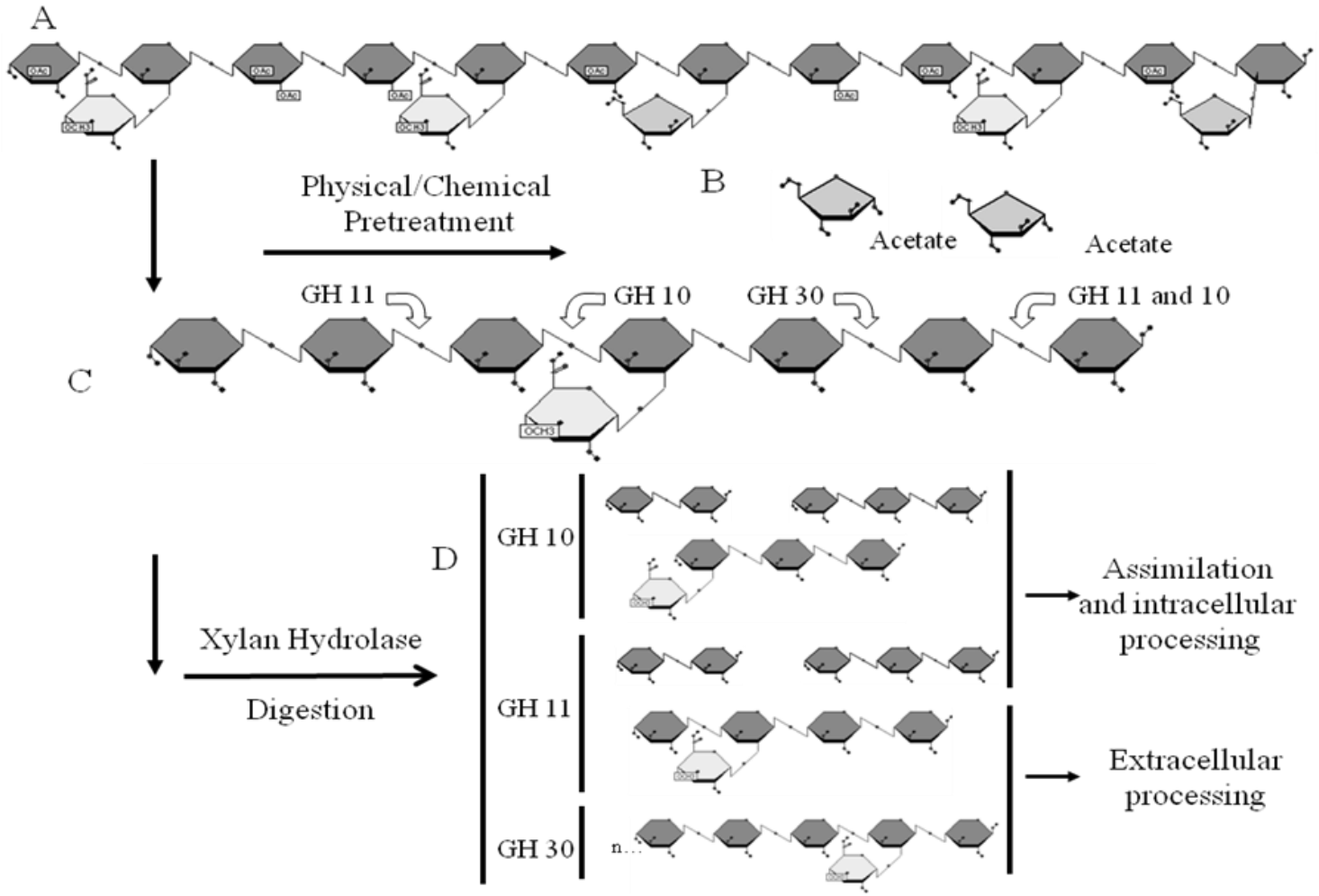
- **Gram-negative *Enterobacter asburiae* E1 (pLOI555) produced ethanol from glucuronoxylan hydrolysate in a theoretical yield of 98% compared to 63% for Gram-negative *Escherichia coli* KO11.**

- **The genetic basis for this metabolic potential may be transferred to *E. coli* and *Klebsiella* biocatalysts for more efficient bioconversion of hemicellulose hydrolysates to biofuels and chemicals.**

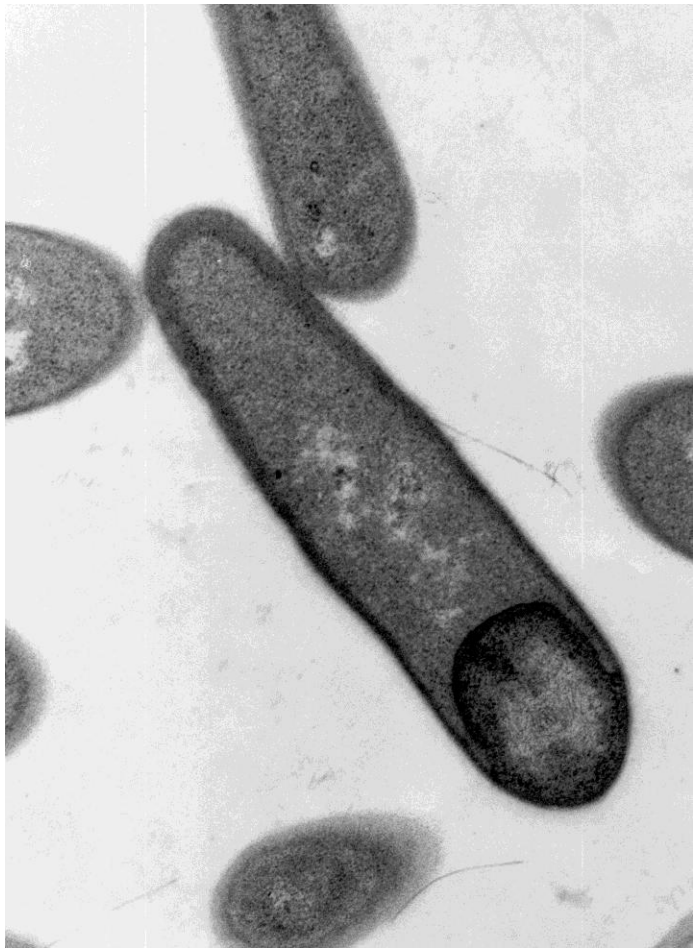
Technologies for Saccharification of Hemicellulose

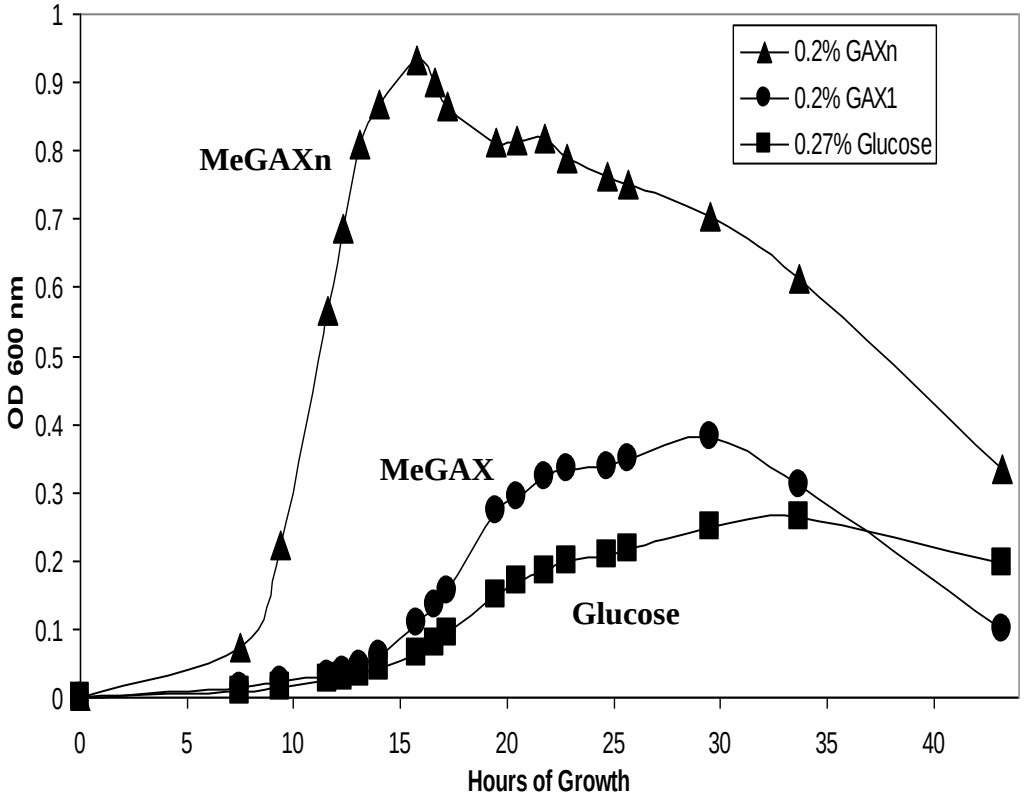
Treatment	Xylose	Aldouronate	Inhibitors
Steam Explosion	X,X2,X3	MeGAX1,2,3	< 0.1% Furfural
Dilute Acid, 140^{o}C	X	MeGAX	< 0.25% Furfural
AFEX, 160^{o}C	Xn	MeGAXn	
0.1 M $Ca(OH)_2$	X2, X3	MeGAX3	
GH10 xylanase			

Pretreatment of O-acetyl-arabinoglucuronoxylan and enzymatic depolymerization of 4-O-methylglucuronoxylan

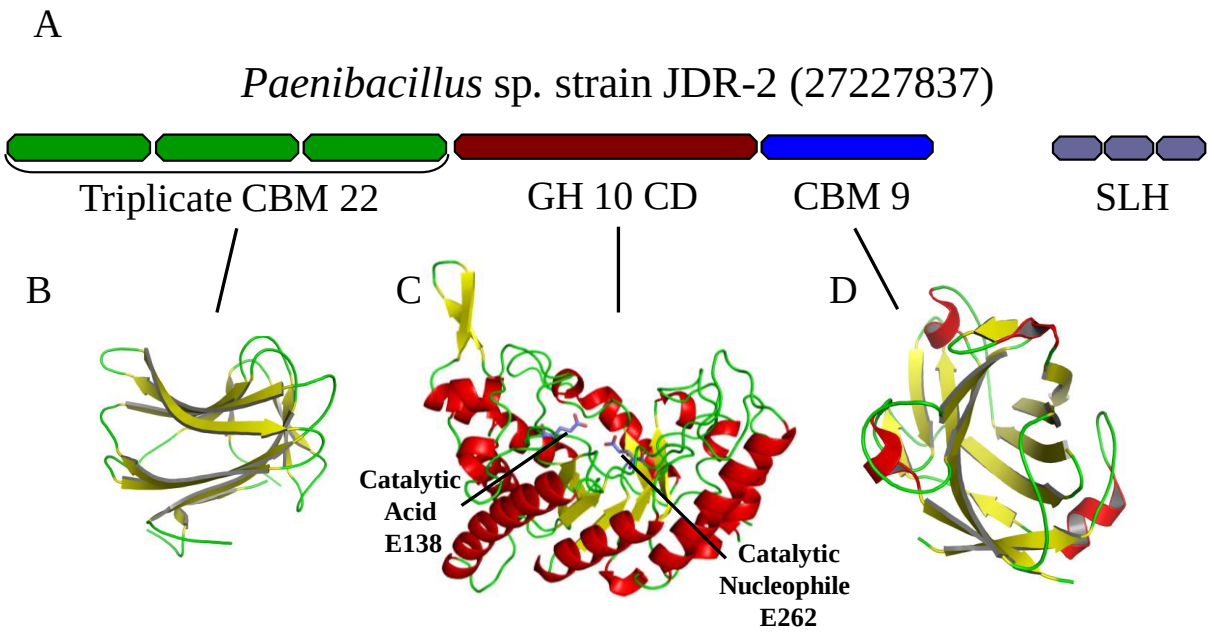


Properties of xylanolytic *Paenibacillus* sp. JDR-2 selected for growth on MeGX

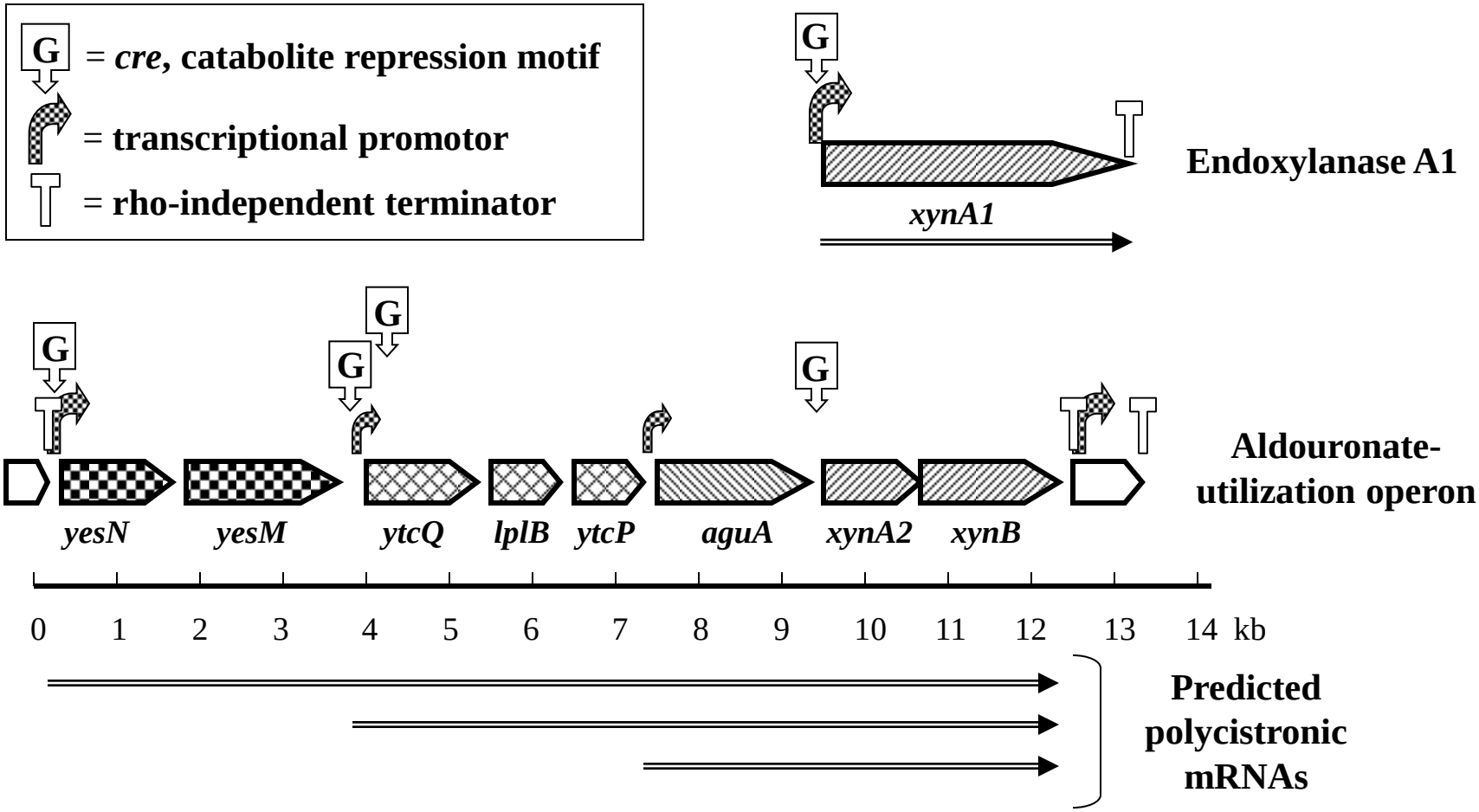




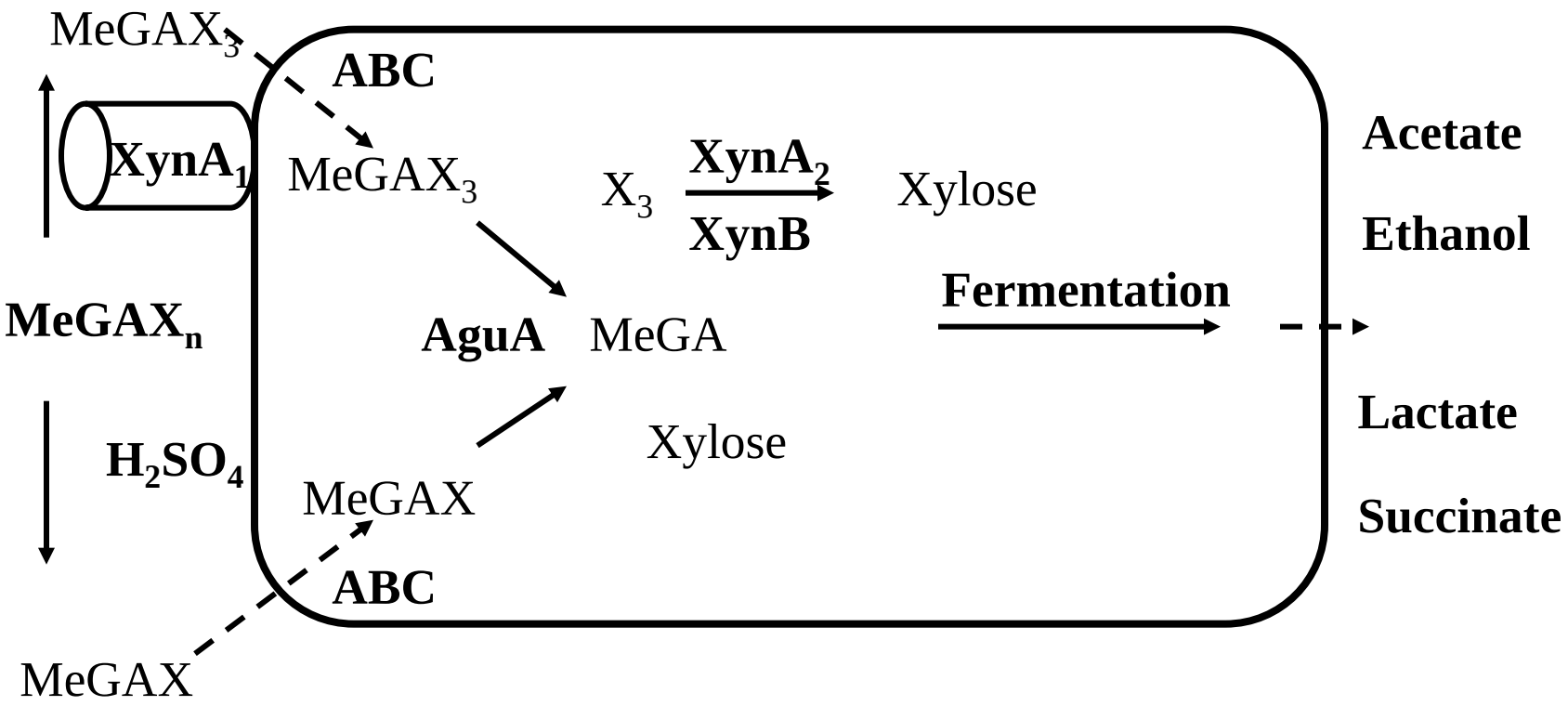
Modular architecture of XynA1 secreted by *Paenibacillus* sp. JDR-2



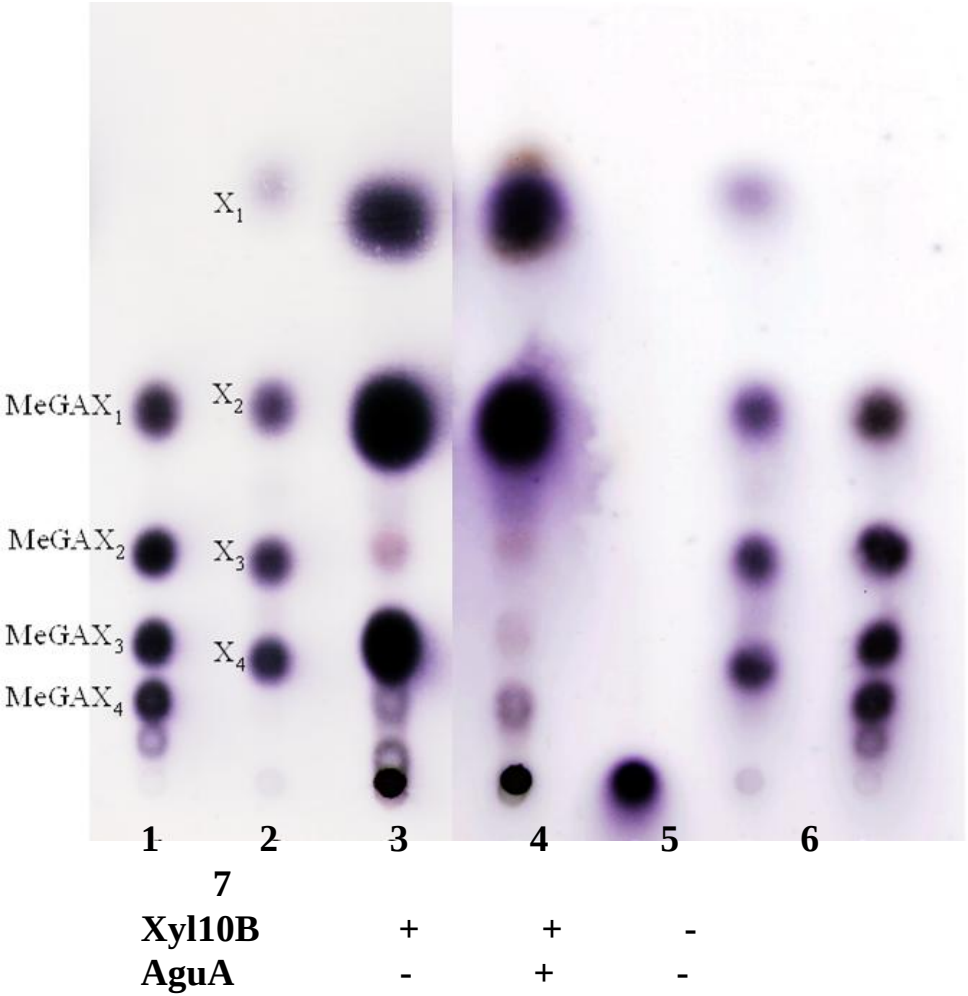
Regulation of expression of aldouronate-utilization genes in *Paenibacillus* JDR-2



Processing of Aldouronic Acids by *Paenibacillus* sp. JDR



Depolymerization of MeGXn with Xyl10B and AguA from thermophilic *Thermotoga maritima*



Lane 1 and 7: standards of $MeGX_{1-4}$
Lane 2 and 6: standards of X_{1-4}
Lane 3: sweetgum with the Xyl10B
Lane 4: sweetgum xylan with Xyl10B and AguA
Lane 5. sweetgum xylan

Summary 2

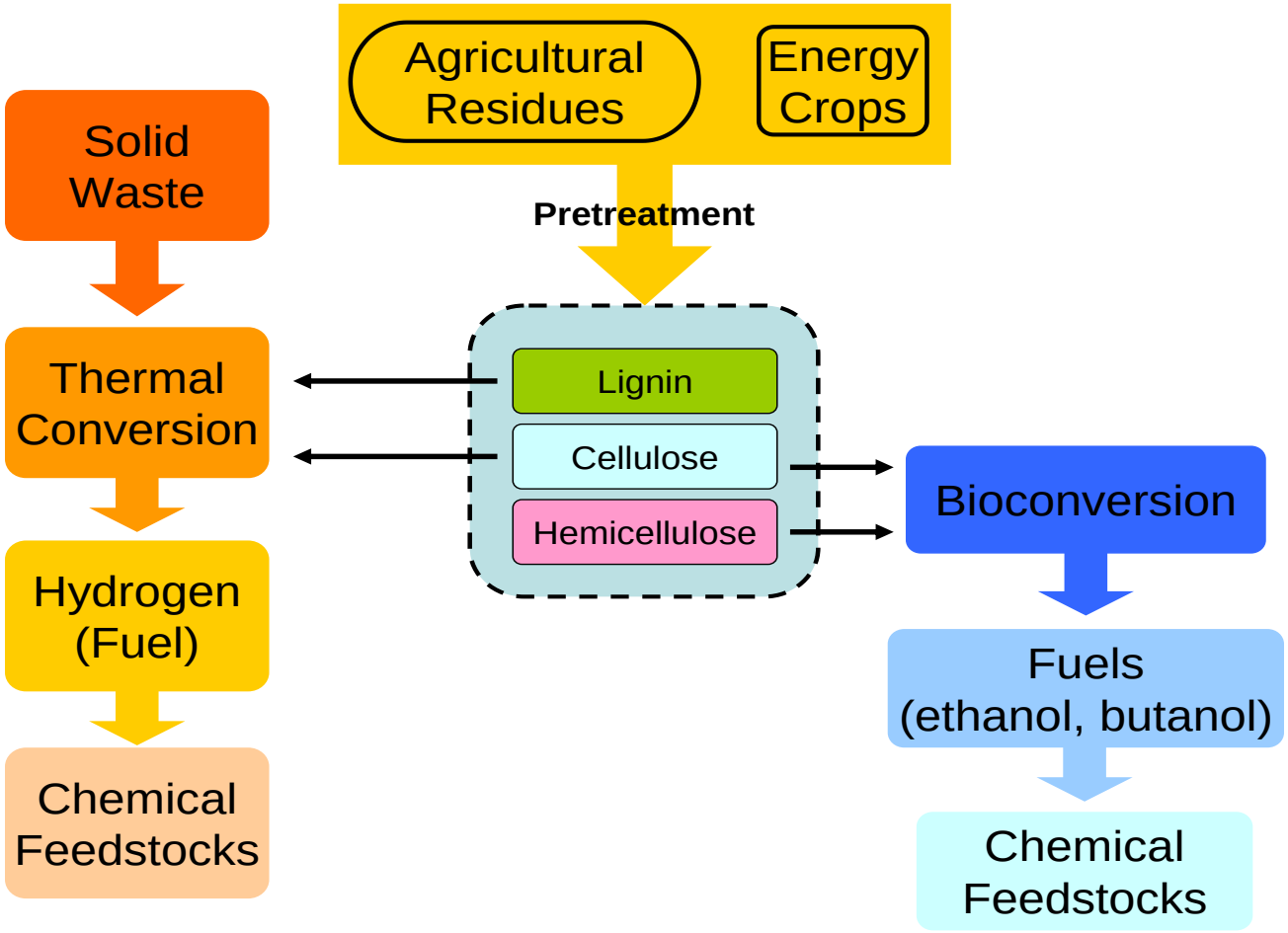
Cell-surface anchored GH10 xylanases catalyze depolymerization of glucuronoxylan and vectoral processing of products.

Bacteria endowed with this capability may be developed for direct conversion of hemicellulosic resources to biobased products

Thermophilic α-glucuronidases and endoxylanases produced in planta may enhance xylan bioconversion by bacterial biocatalysts.

These systems may allow the application of alkaline pretreatment protocols to reduce costs and increase yields of fuels and chemicals from agicultural residues and energy crops.

Options for Biomass and Solid Waste Conversion to Energy



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