Hydrogenomics of the Extremely Thermophilic Microorganism *Caldicellulosiruptor saccharolyticus* for Bioenergy Production

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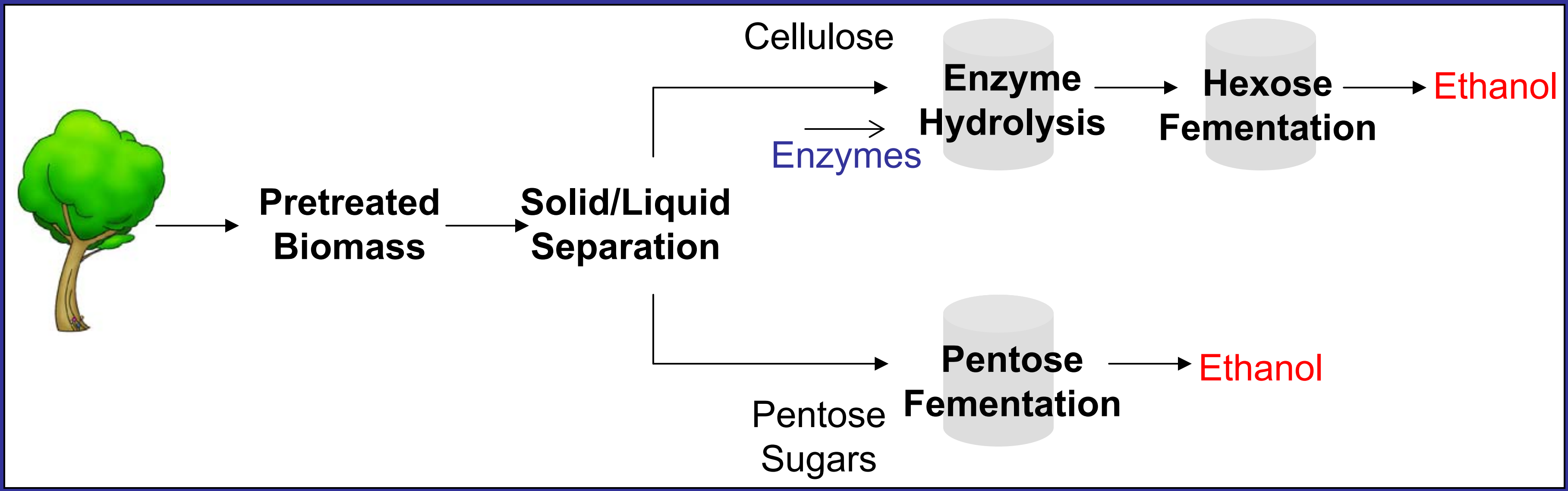
Biohydrogen

Types of Biomass

Switchgrass

Poplar

- Sources of cellulose more abundant than corn



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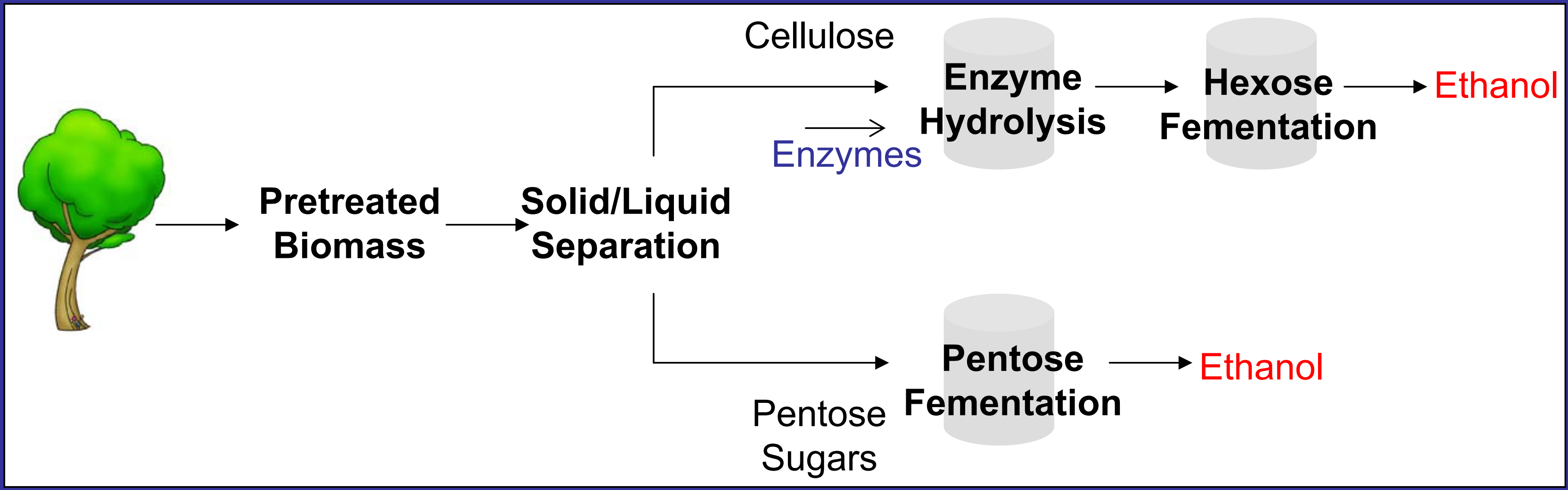
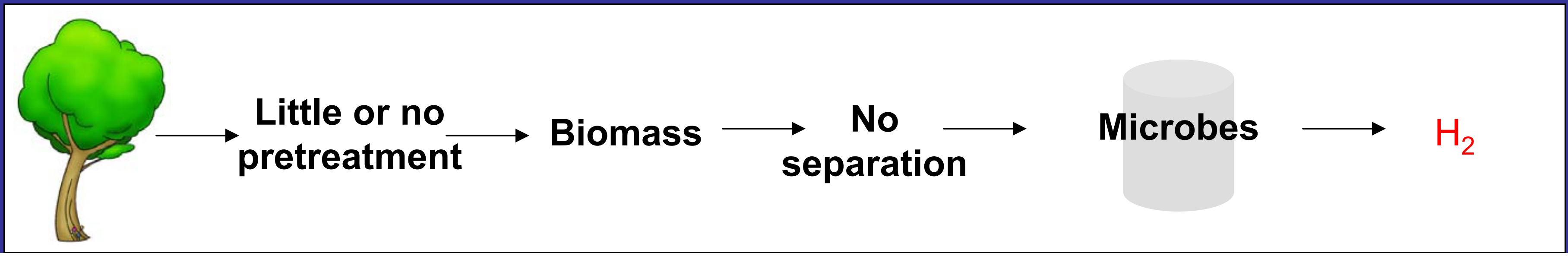
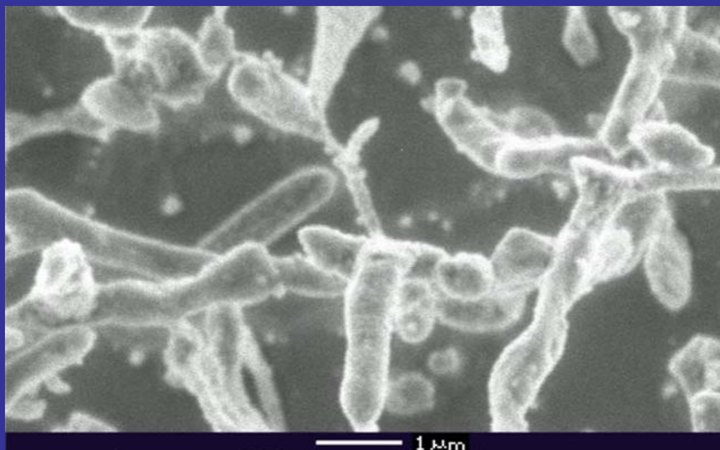




Caldicellulosiruptor saccharolyticus



Downstream from a 78^{0}C pool

T_{opt} = 70^{0}C

Fermentative heterotroph

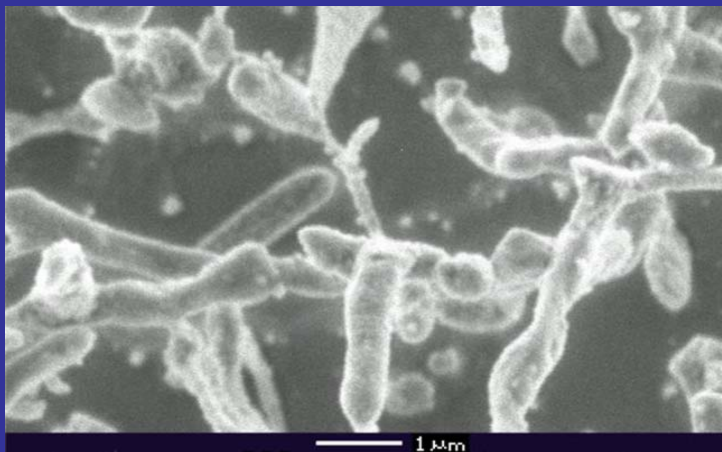
<u>Bacterium</u>

Rod-shaped

Asporogenous

Produces H_2
acetate, lactate, CO_2 and
ethanol

Caldicellulosiruptor saccharolyticus



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Fermentative heterotroph

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Oligo/Monosaccharides

Grows on:
Cellobiose
Fructose
Galactose
Glucose
Lactose
Maltose
Rhamnose
Xylose

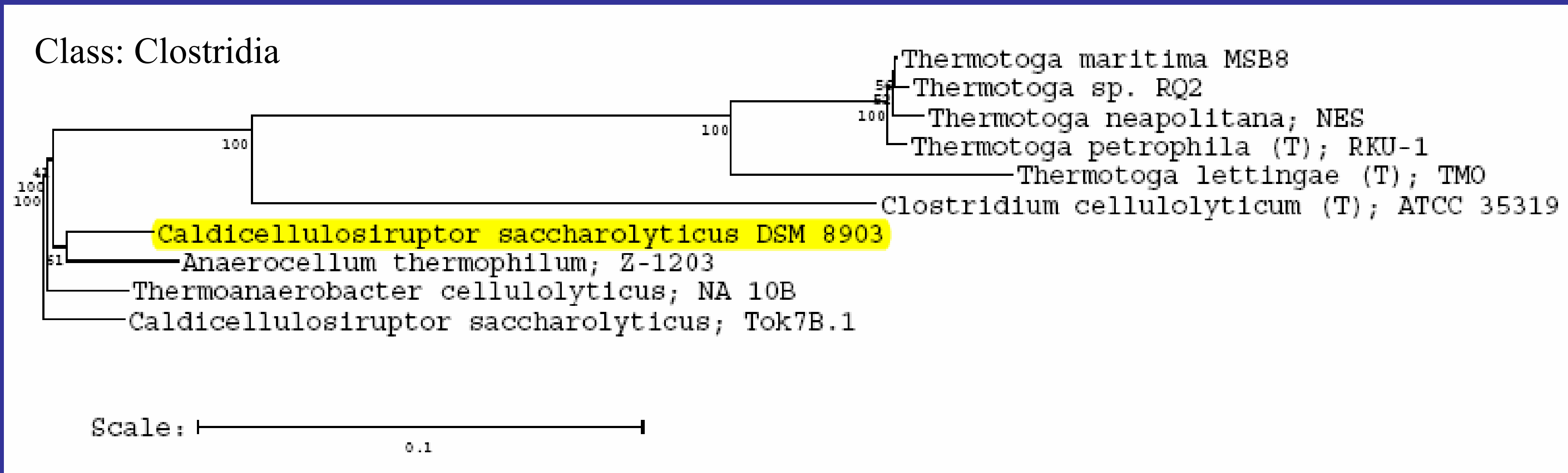
Does not grow on:
Sorbitol
Raffinose
Ribose
Sorbose

Polysaccharides

Grows on:
Cellulose (Avicel)
Starch
Pullulan
Laminarin
Lichenin
Pectin
Xylan

Does not grow on:
Inulin
Glycogen

Caldicellulosiruptor saccharolyticus

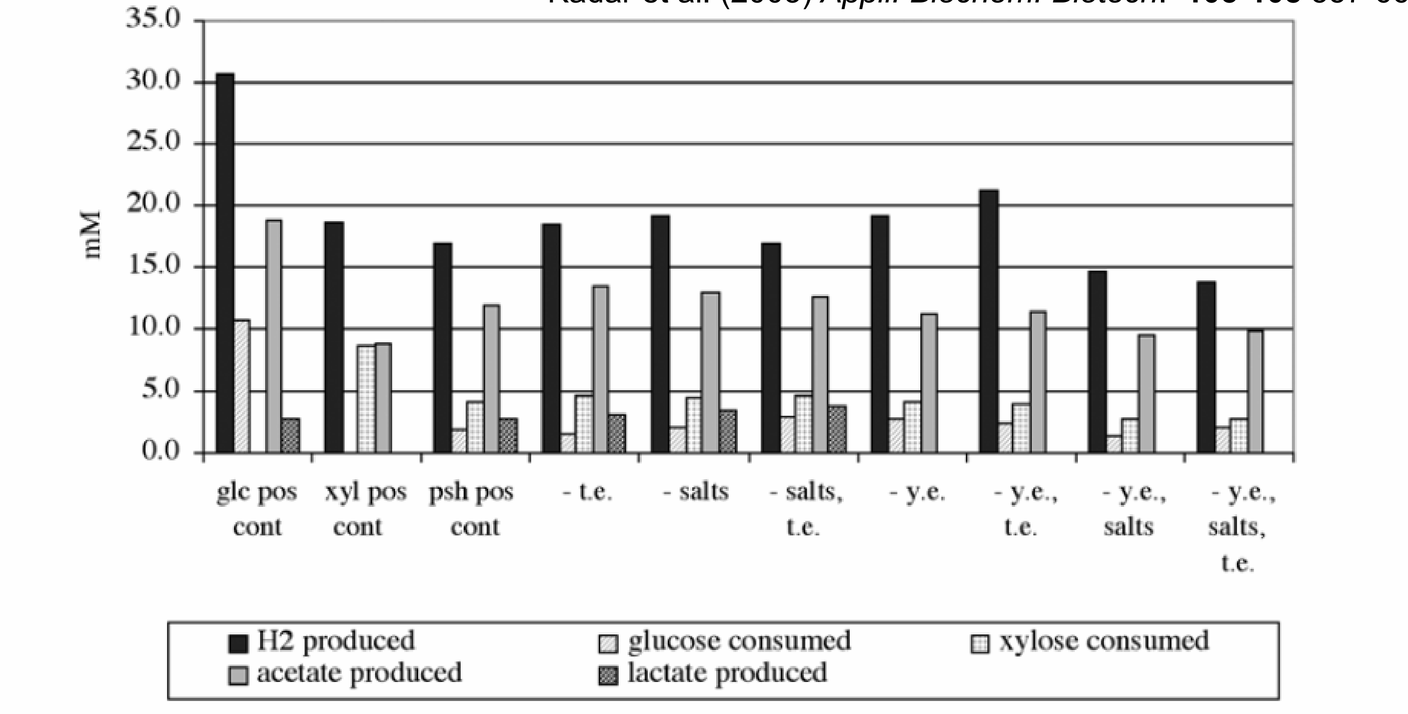


- Thermophiles offer many advantages over mesophiles
  - –Increased solubility of substrates
  - –No problems with sulfate reducers and methanogens
  - –Fewer fermentative byproducts
  - –Approach Thauer limit: $4H_2$ / glucose

$BioH_2$ Production by *Csac*

Grows on waste paper hydrolysate

Kadar et al. (2003) *Appli. Biochem. Biotech*. **105-108** 557-66.



Higher tolerance H_2 than other thermophiles

BioH$_2$ Production by *Csac*

Grows on waste paper hydrolysate

- Co-ferments xylose and glucose
- Xylose > Glucose

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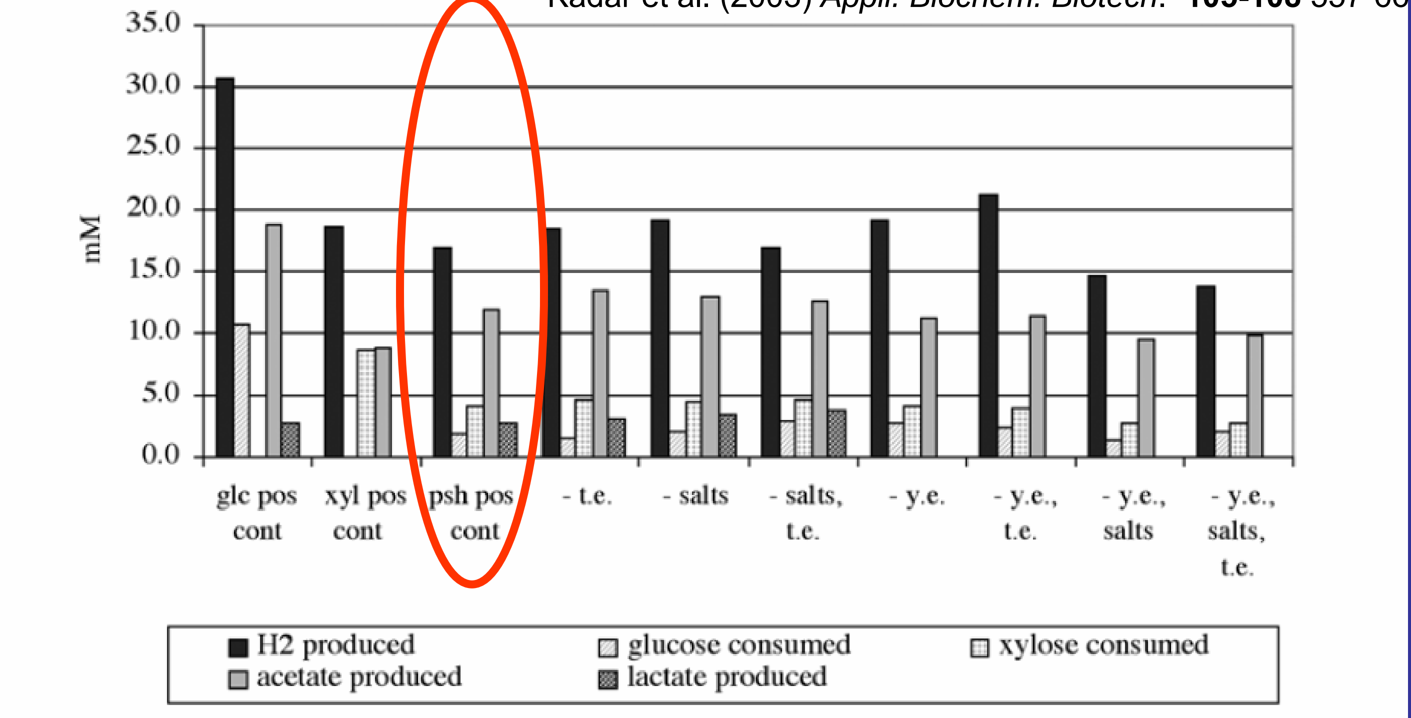

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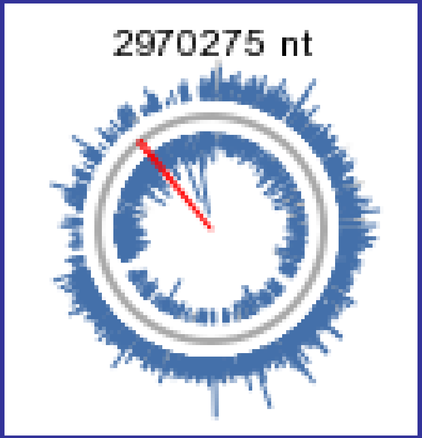
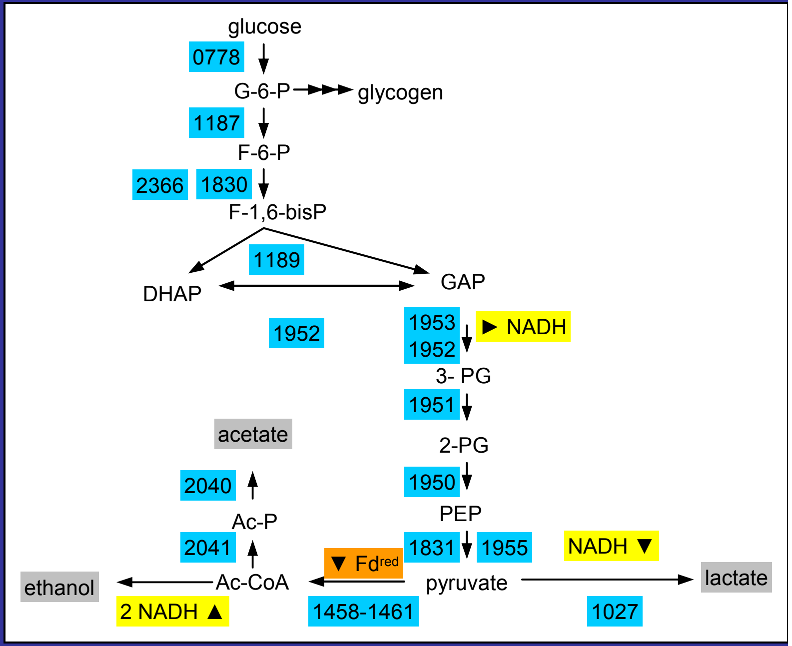
Csac Genome



- Proposed sequencing to JGI Spring 2006
  - Draft genome in 136 contigs Fall 2006
  - Sequence finished Feb 2007
- 2.97 Mb with a 35.3% GC content
- 2793 ORF's identified
  - > 60 GHs found in the genome
  - Contains > 177 ABC transporter genes
  - A fructose-specific PTS system
  - EM pathway
  - Non-oxidative PPP



Csac Glycoside Hydrolases

Enzyme	GH family	MW (kDa)	Locus
pullulanase (pulA)	13	95.7	Csac0671
β-mannanase	5 and 44	146.9	Csac1077
cellulase (CelA)	9 and 48	193.7	Csac1076
cellulase (CelB)	10 and 5	117.6	Csac1078
β-xylosidase (XynB)	39	56.4	Csac2404
endo-1,4-β-xylanase	10	36.5	Csac2405
acetyl esterase (ORF2)	-	30.6	Csac2407
xylanase (XynA)	10	40.5	Csac2408

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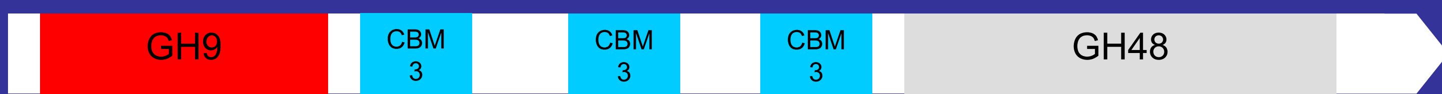
CelA



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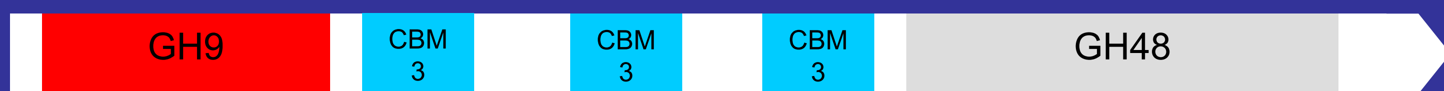
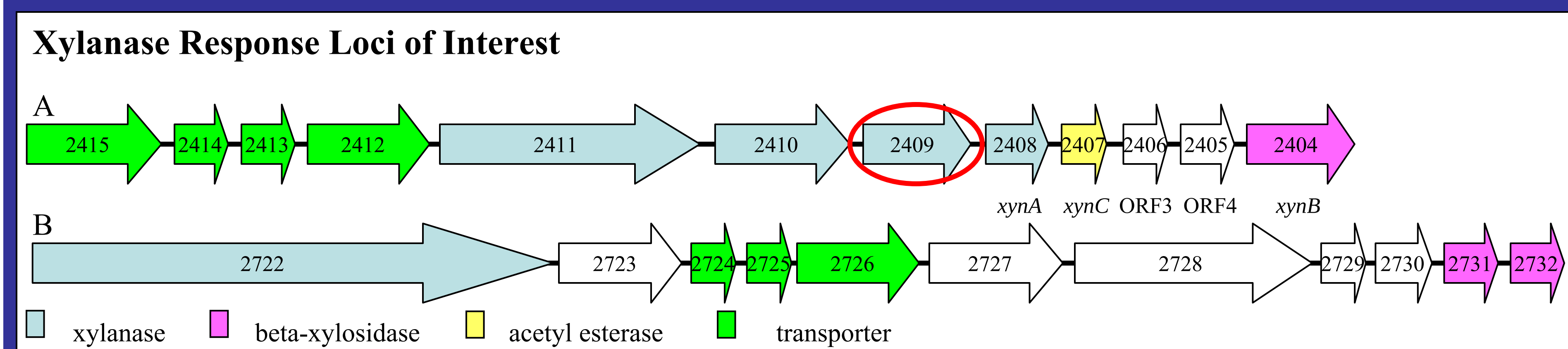
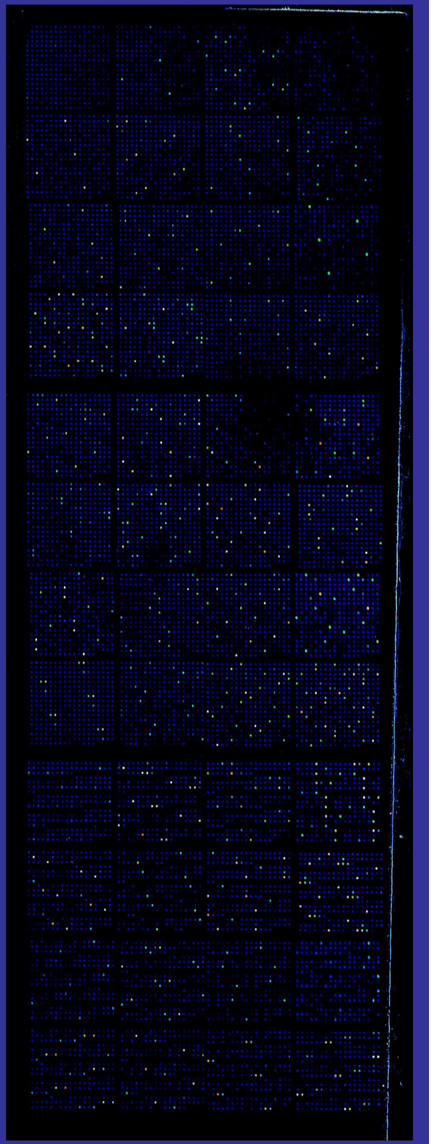




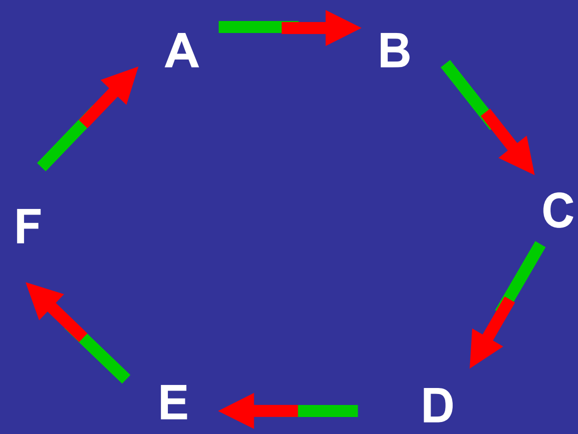
Csac Transcriptomics

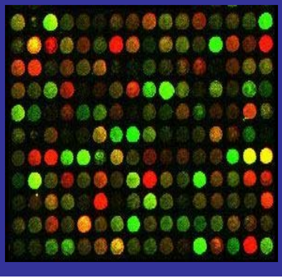
Csac oligonucleotide spotted microarray

- 2793 ORFs represented by 60-mer probes
- 5 copies of each probe, randomized location



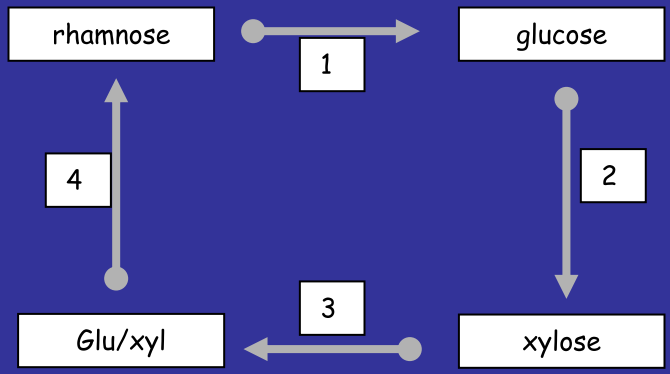
Loop experimental design





→ **Data analysis: Mixed Model ANOVA** → **Transcriptional Response**

Csac Xyl/Glu Co-fermentation



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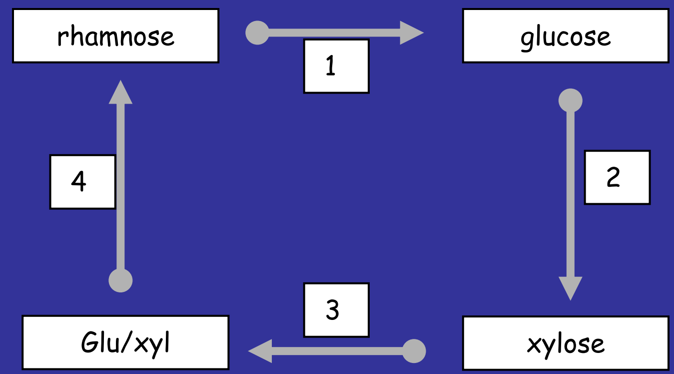

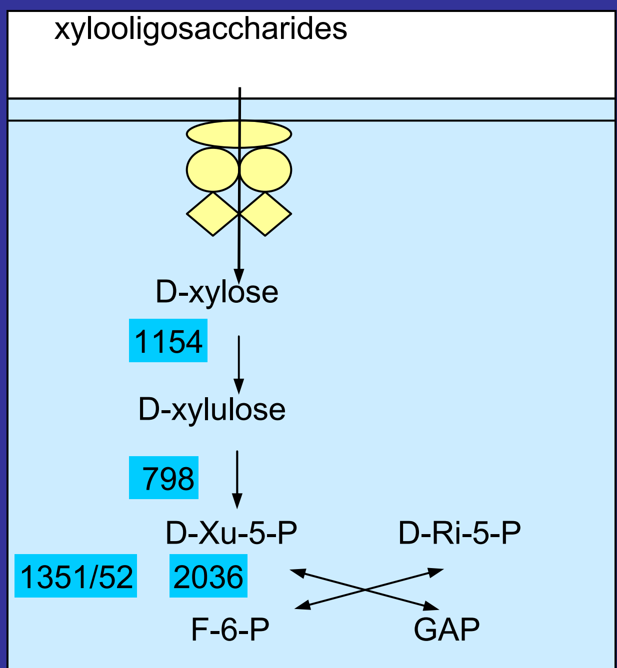

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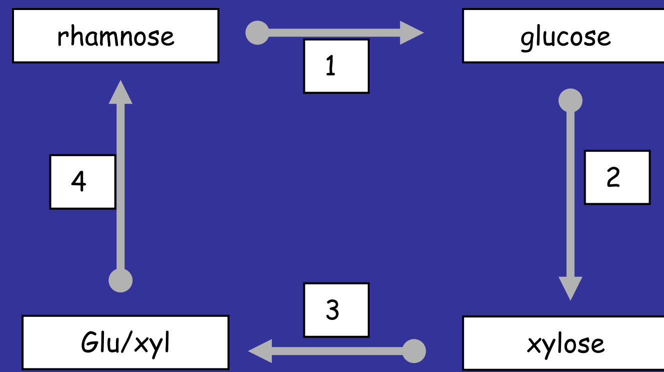
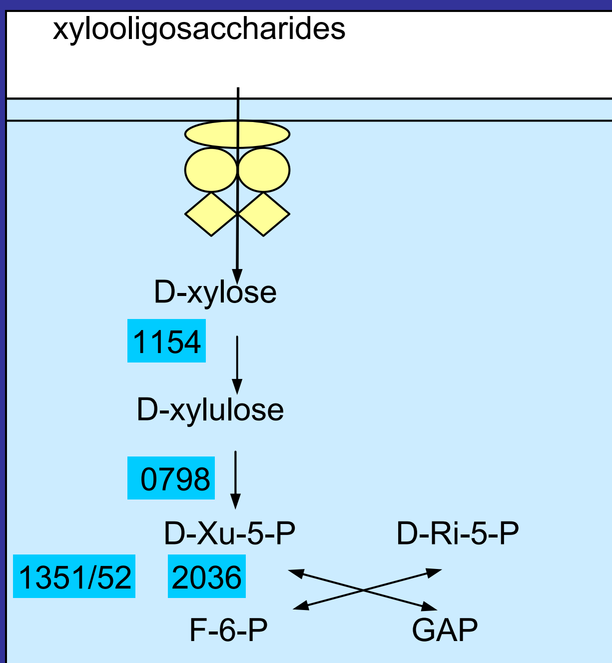
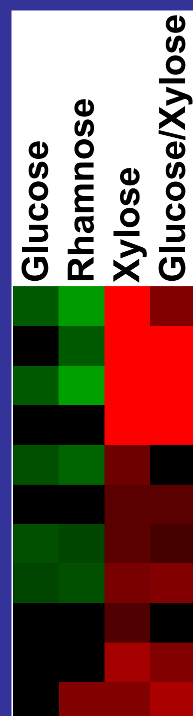


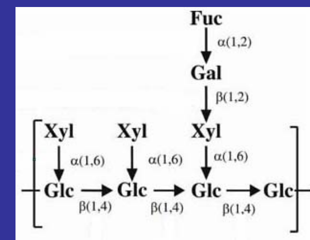


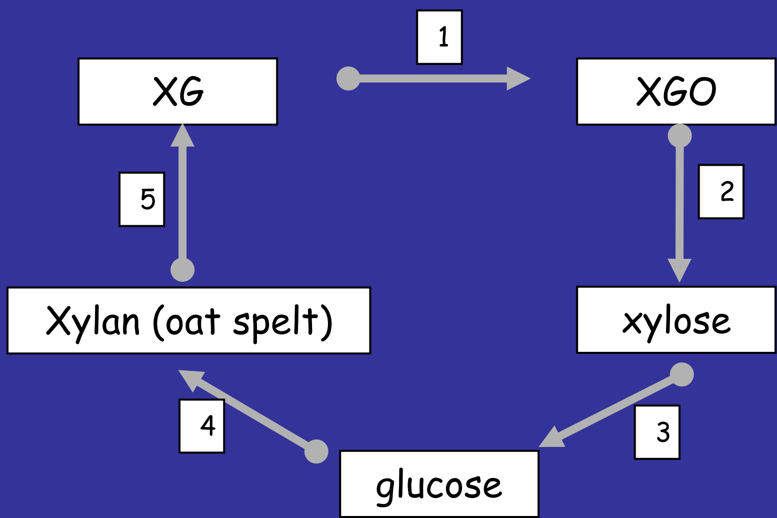


Gene ID	Annotation
Csac0240	ABC transporter
Csac0241	ABC transporter transmembrane
Csac0242	ABC transporter SBP
Csac0692	ABC transporter SBP
Csac0693	ABC transporter permease
Csac0694	ABC transporter transmembrane
Csac0695	Xylose repressor
Csac0696	Endo-1,4-beta-xylanase
Csac0798	Xylulokinase
Csac1154	Xylose isomerase-like
Csac1351	Transketolase

XGO Utilization

xyloglucan (XG) links cellulose microfibrils together in plants



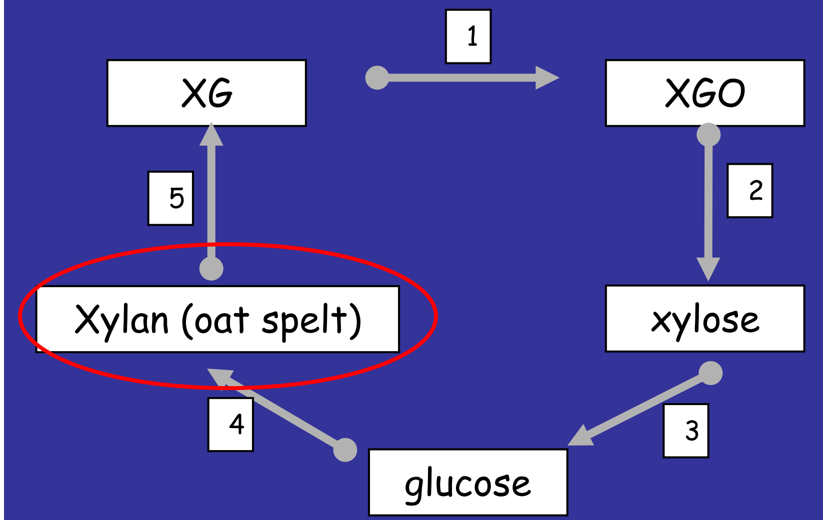


XG differently transcribed ORFs				
ORFs	XG vs XGO	XG vs xylose	XG vs glucose	XG vs xylan
↑	305	187	103	101
↓	438	181	73	39

Up-regulation of genes to degrade XG side chains and backbone

Fold changes of GH ORFs up-regulated on XG				
Gene	Annotation	XG vs XGO	XG vs glucose	XG vs xylose
Csac0689	pullulanase type I	2.6	2.1	2.3
Csac0779	acetyl xylan esterase	8.8	No change	No change
Csac0784	1,4-α-glucan branching enzyme	4.2	No change	No change
Csac1078	Endoglucanase	4.4	3.7	3.5
Csac1080	β-mannanase	3.4	3.7	2.6
Csac1118	α-galactosidase	3.5	4.0	5.5

Xylan Utilization



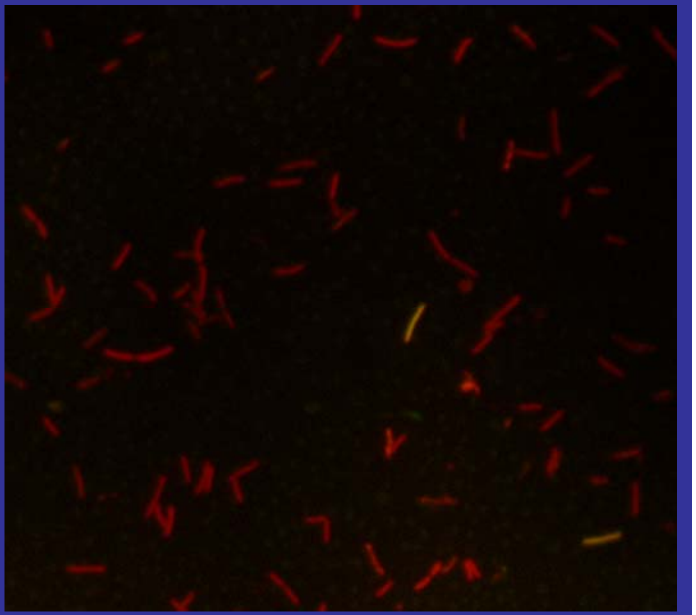
Xylan differently transcribed ORFs			
ORFs	xylan vs XGO	xylan vs xylose	xylan vs glucose
⬆	231	172	168
⬇	91	70	233

- Transcription of other hemicellulose degrading genes
  - β-galactosidases, β-mannosidases, endoglucanases and pullulanases
- Constitutive expression of xylanases
  - Also found reported in fungi and the bacterium *Streptomyces cyaneus*

Csac Biomass Degrader

Enzymes used synergistically to break down biomass material

Co-culture with a cellulose degrader to ferment sugars to biofuels



Acknowledgments

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WHERE DISCOVERIES BEGIN

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Collaborators:

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Ward lab at Genencor, Palo Alto CA
Adams lab at UGA, Athens GA

Kelly Group Members:

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Jaspreet Notey	J. Morgan Harris	Steven Gray
Inci Ozdemir		

<u>Past Students and Post-Docs</u>