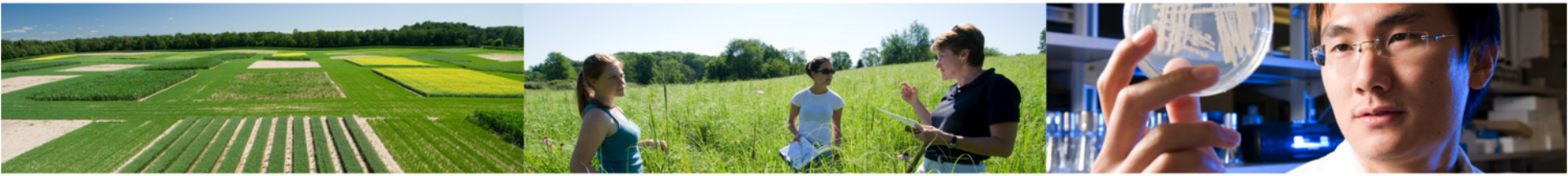
GREAT LAKES BIOENERGY RESEARCH CENTER



BERAC Update

Timothy Donohue

Director, Great Lakes Bioenergy

Professor of Bacteriology, University of Wisconsin-Madison

February 27, 2015

Systems Analysis of Biomass & Biofuel Production

Cropping Systems	Pretreated Biomass	Hydrolysate	Biofuels
Measurables			
Site/Soil Type Crop/Seed/Row Plant/Harvest Date Fertilizer/Herbicide Season/Weather	Cellulose Hemicelluloses Lignin Plant Cell Residue	Total CHO C-5 & C-6 Sugars Amino Acids Organic Acids/Amides Ammonia/Phosphate ~30 Metals/Inorganic Ions	Hydrolysate Inputs Transcripts Targeted Metabolites Excreted Products Fuel Input/Output COD Microbial Growth

Data Management & Analysis

Scientific Discoveries

Collaboration Enables Discoveries

GLBRC

DOE BER

BESC BioEnergy Science Center

jbei Joint BioEnergy Institute

KBASE predictive biology
DOE Systems Biology Knowledgebase

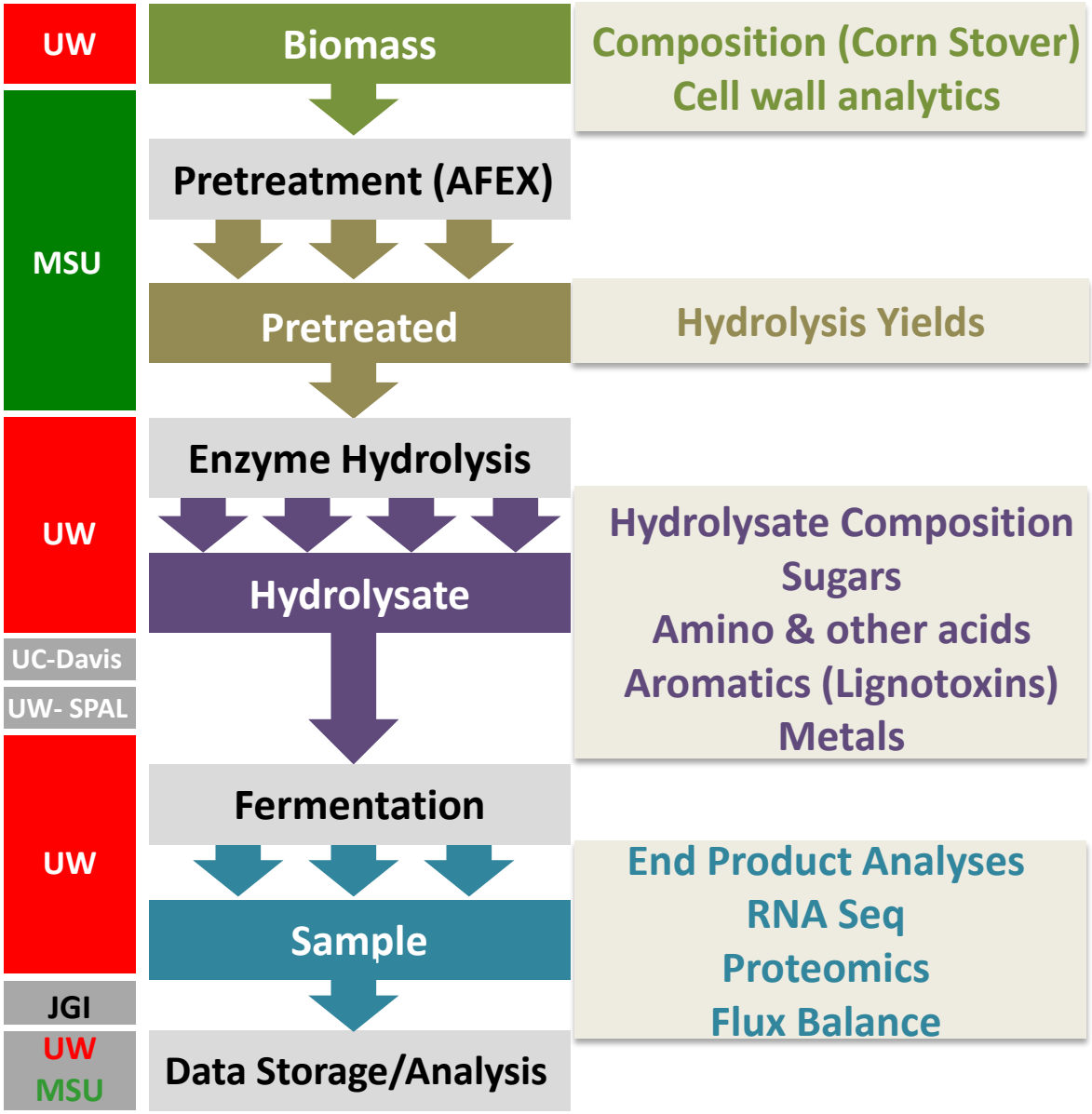
JGI DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

"External"

USDA LTER FOREST SERVICE US DEPARTMENT OF AGRICULTURE NRCS U.S. FISH & WILDLIFE SERVICE ENGINE RESEARCH CENTER UNIVERSITY OF WISCONSIN - MADISON est 1946 PRL

Farmers
Landowners
Universities

Biomass Field to Fuel Analysis



Chemical composition of hydrolysates

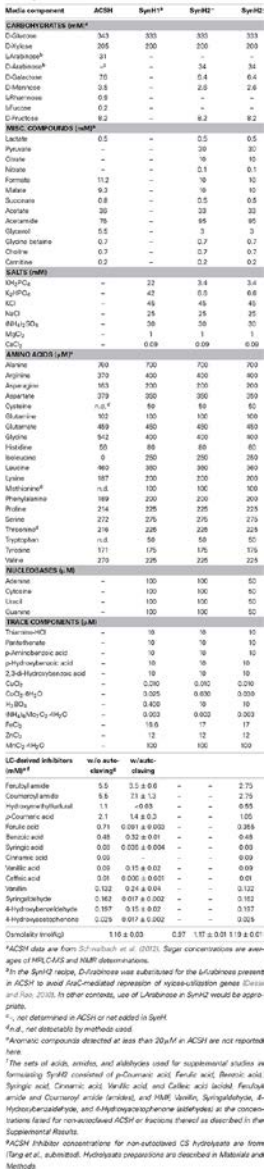
Carbohydrates

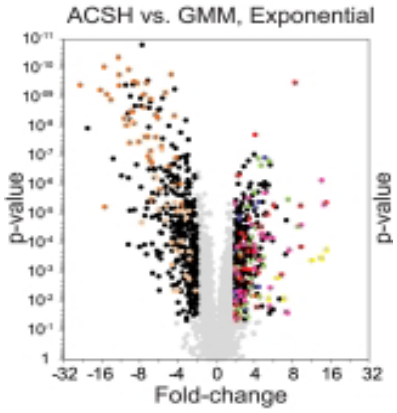
(Glc, Xyl, Ara, Gal, Man, Rha, Fru, Fuc)

AAs (-C,M,W)

Nucleosides, Organic Acids, Aldehydes, Ketones, Amides Aromatics

Metals, Minerals & Anions



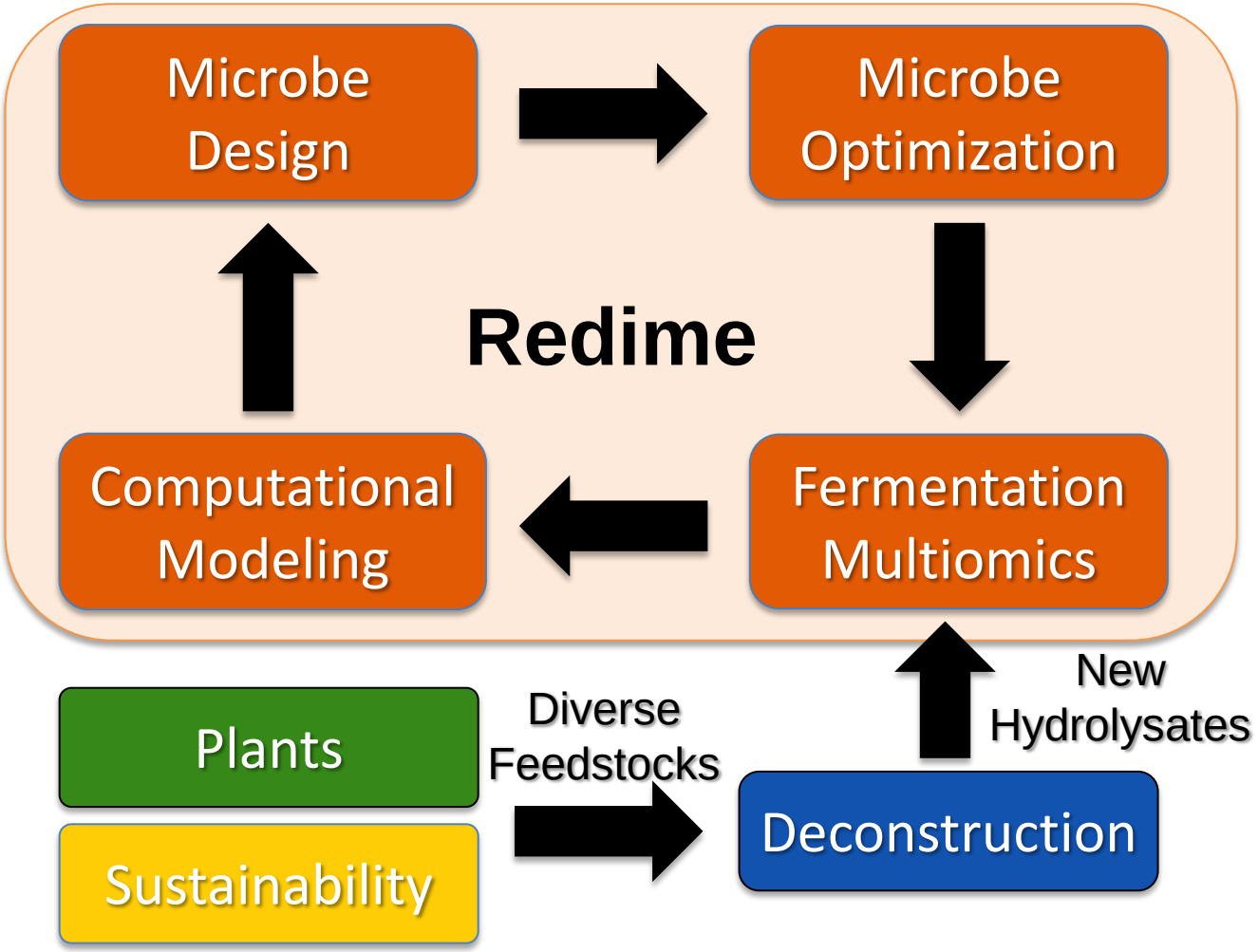


- Stress related
- Efflux pumps
- Anaerobic respiration
- Carbohydrate use (non-glucose)
- Amino acid synthesis
- Flagella,Chemotaxis
- Thiamine synthesis
- Iron metabolism
- Citrate lyase
- Osmotic tolerance

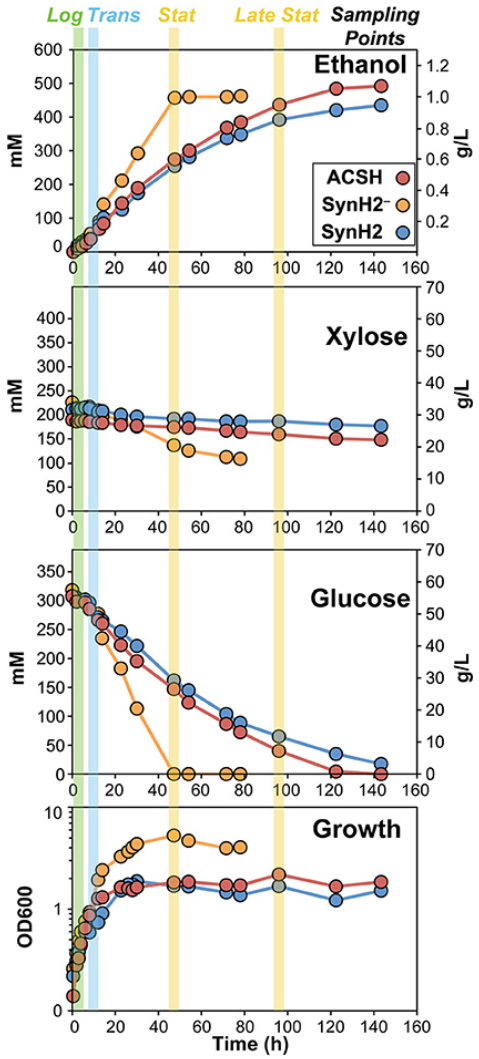
Keating, *et al*. 2014 *Frontiers Micro.* 5:402

Schwalbach, *et al.* 2012 *App. Env. Micro.* 78:3442

Reiterative microbial design targets sites for strain improvement



Hydrolysate composition negatively impacts biofuel fermentations

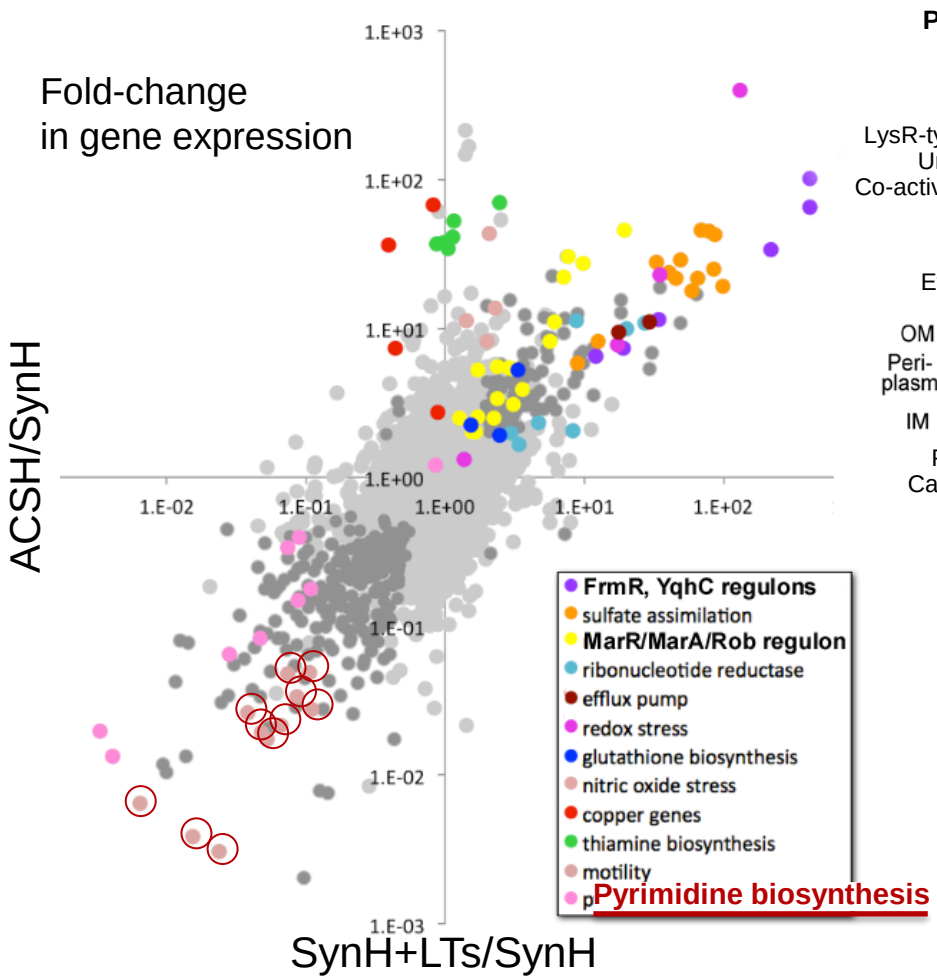


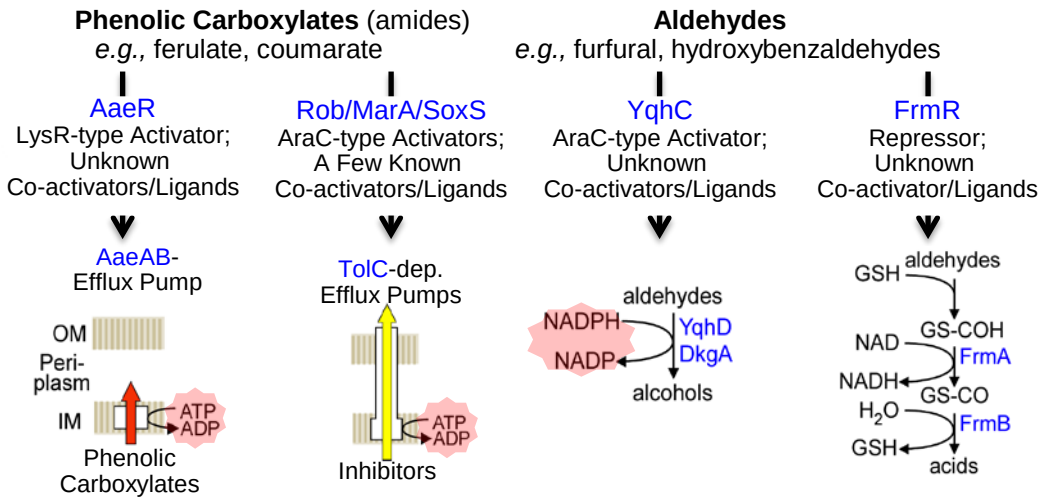
- Negative impact of ACSH on growth/biomass production, xylose and glucose utilization & ethanol production
- Recapitulate behavior with synthetic hydrolysate (SynH2)
- Negative impact overcome in SynH2$^-$ (SynH2 minus aromatics from ACSH)

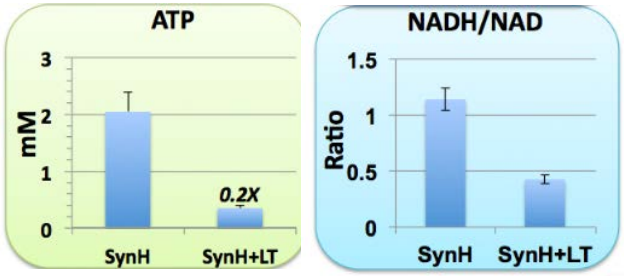
Lignotoxins (LTs)

5.5 mM Feruloyl amide
5.5 mM Coumaroyl amide
2.1 mM Coumaric acid
0.5 mM Benzoic acid
1.1 mM Hydroxymethylfurfural
0.7 mM Ferulic acid
8 minor LTs (<0.5 mM)

LTs induce energy-consuming pathways & deplete ATP/NADH

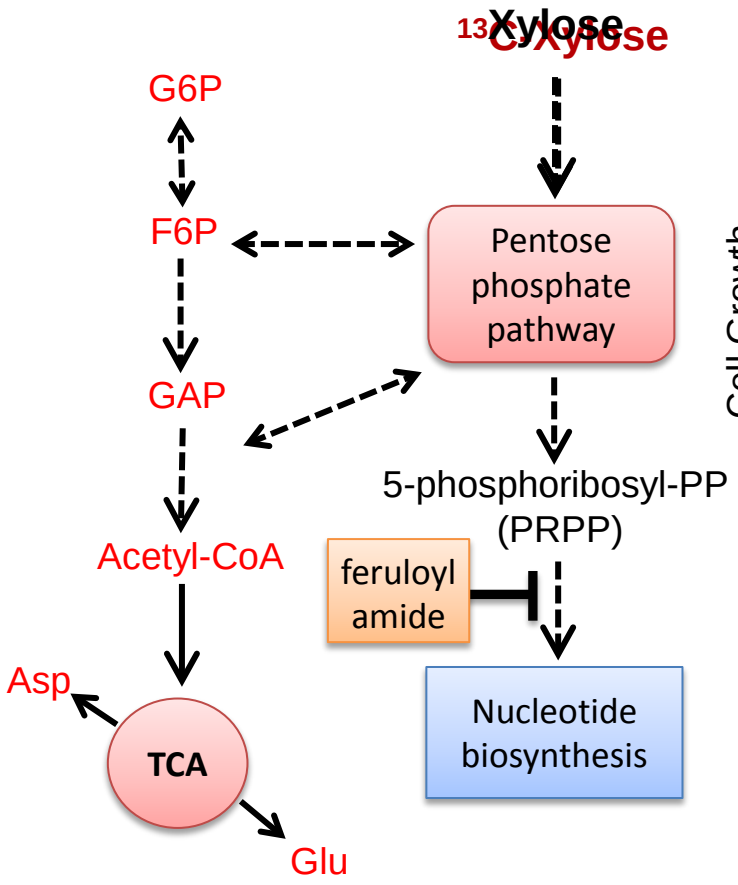




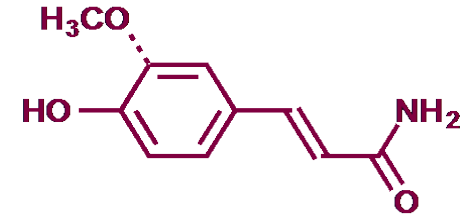


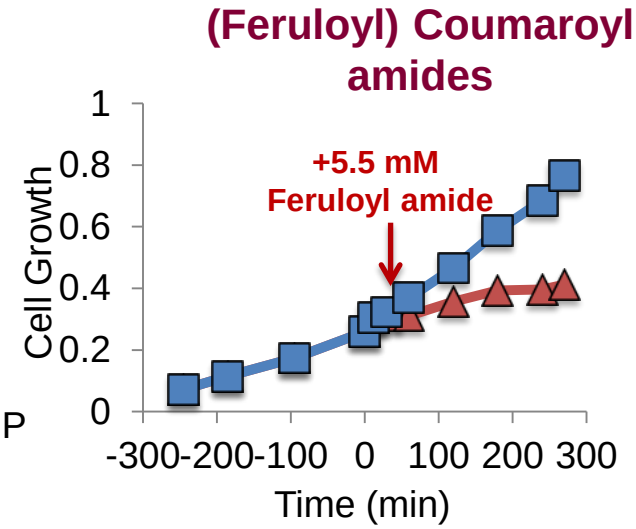
Keating, *et al*. 2014 *Frontiers Micro.* 5:402

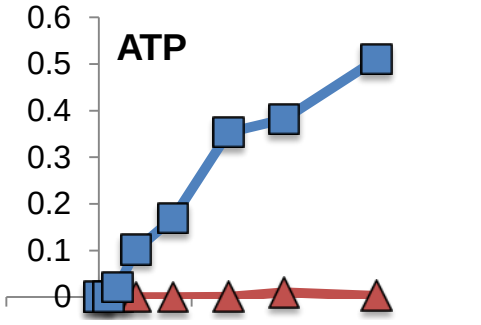
Feruloyl amide inhibits growth, increases PRPP pools & blocks nucleotide synthesis

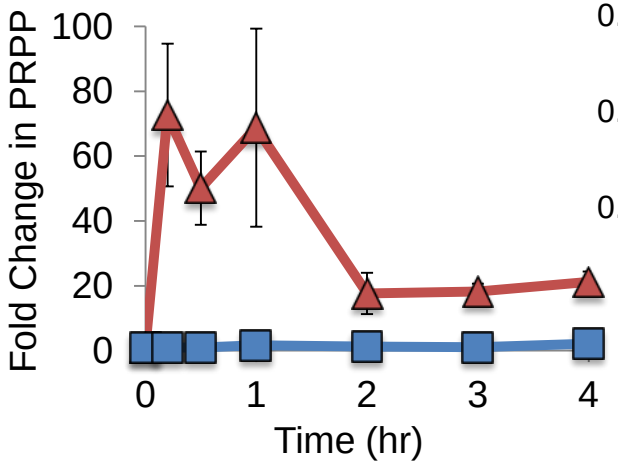


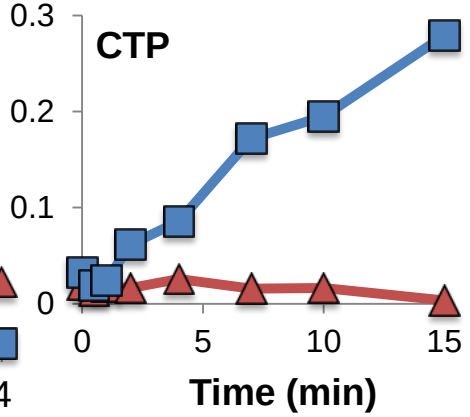
Amador-Noguez, *et al. in preparation*



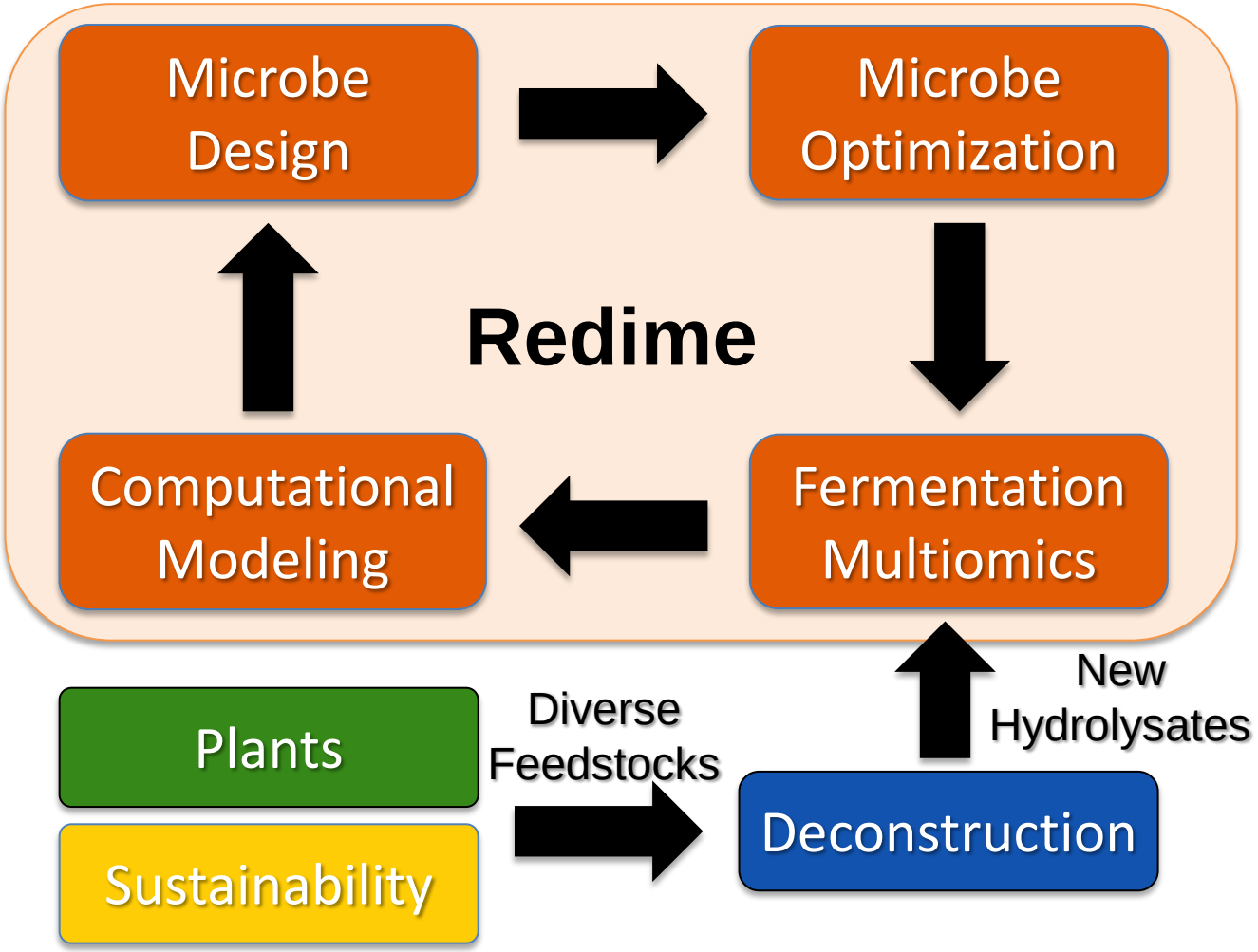




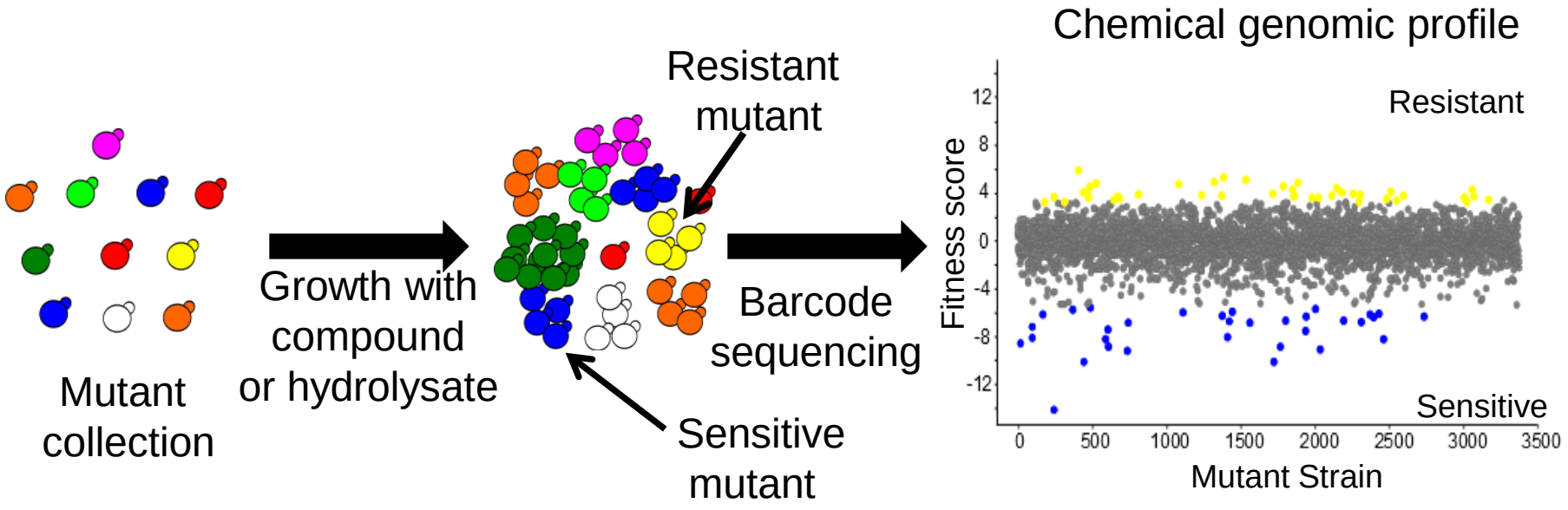




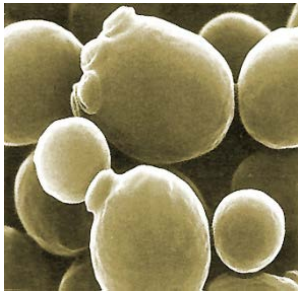
Reiterative microbial design targets sites for strain improvement



Genomic fingerprinting of inhibitors

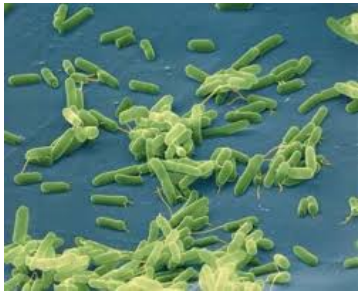


S. cerevisiae



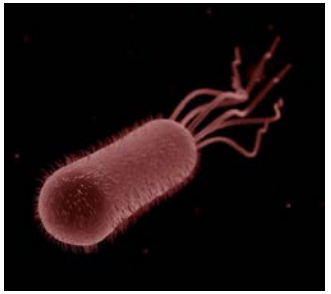
Parsons, *et al*. 2006
Piotrowski, *et al*. *in press PNAS*

Z. mobilis



Skerker, *et al*. 2013

E. coli

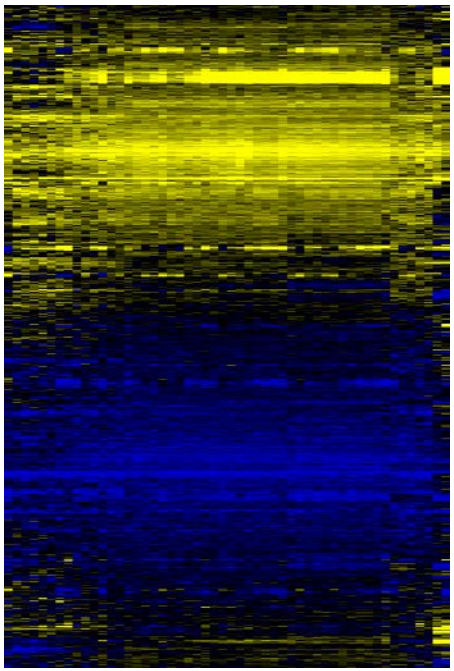


Otsuka, *et al*. 2015

Conserved mode of action of LT's

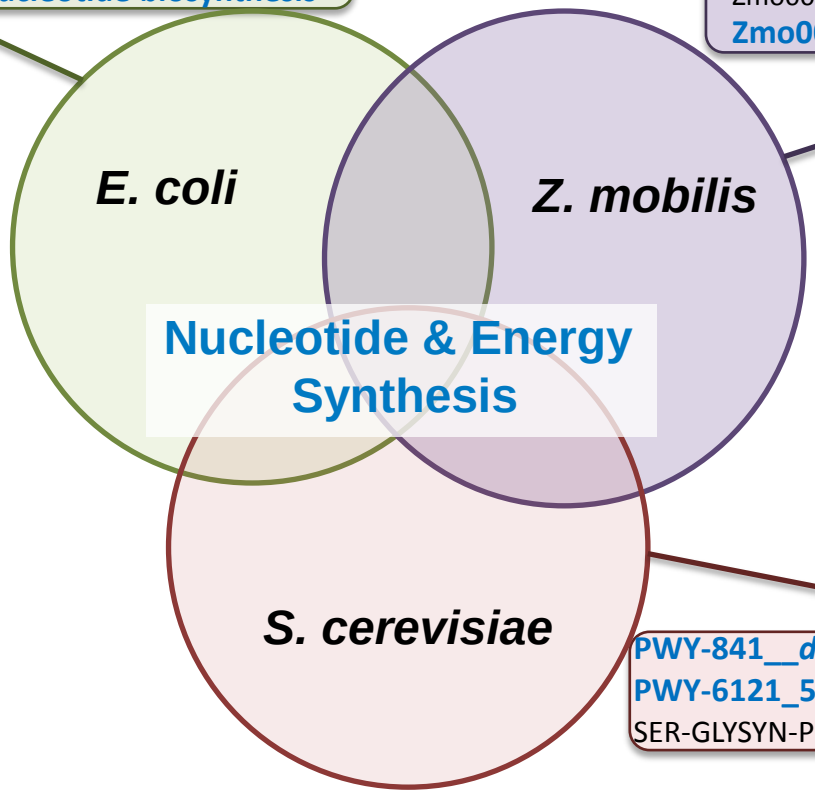
Chemical genomic analysis of 29 hydrolysate LT's against 3 "disparate" microbes (*E. coli*, *Z. mobilis*, *S. cerevisiae)*

- Nucleotide & energy metabolism responsive across inhibitors & microbes



PWY0-1334__electron transfer from NADH to cytochrome *bd* oxidase
PURINE2-PWY__*de novo* purine nucleotide *synthesis*
PWY-7219__*de novo* adenosine nucleotide biosynthesis

Zmo00290 Valine, leucine and isoleucine biosynthesis
Zmo00340 Histidine metabolism
Zmo00230 Purine metabolism



E. coli: Otsuka, *et al*. 2015
Z. Mobilis: Skerker, *et al*. 2013

PWY-841__*de novo* purine biosynthesis
PWY-6121_5-aminoimidazole ribonucleotide synthesis
SER-GLYSYN-PWY__Serine & glycine synthesis

Systems Analysis of Biomass & Biofuel Production

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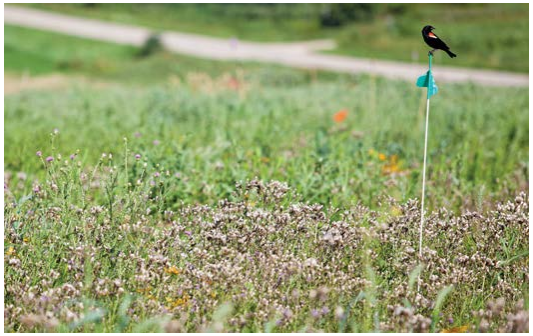
Data Management & Analysis

Scientific Discoveries

Systems Analysis of Biomass & Biofuel Production



Analysis of additional biomass crops

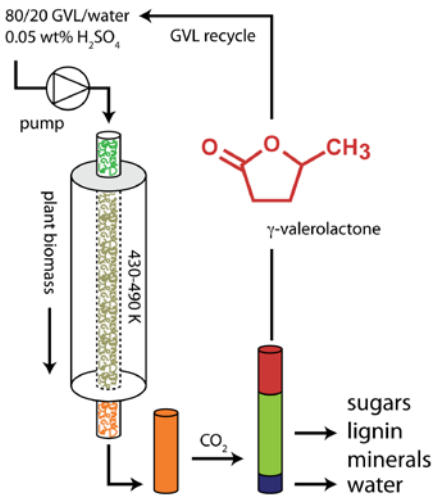
- Poplar
- Miscanthus
- Switchgrass
- Native Prairie
- Mixed Feedstocks

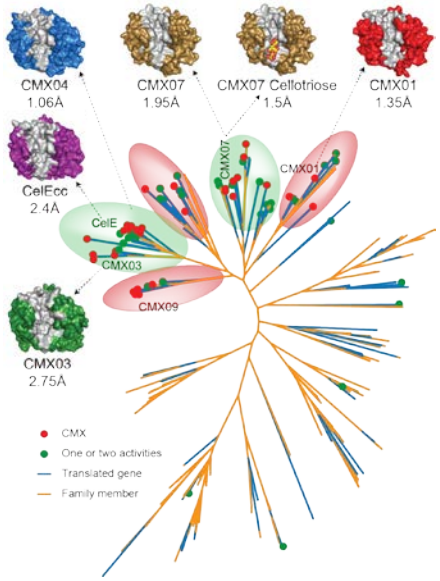
- Assess yearly/regional feedstock variations
- Impact of biomass trait modifications (Zip-Lignin & others)

Systems Analysis of Biomass & Biofuel Production

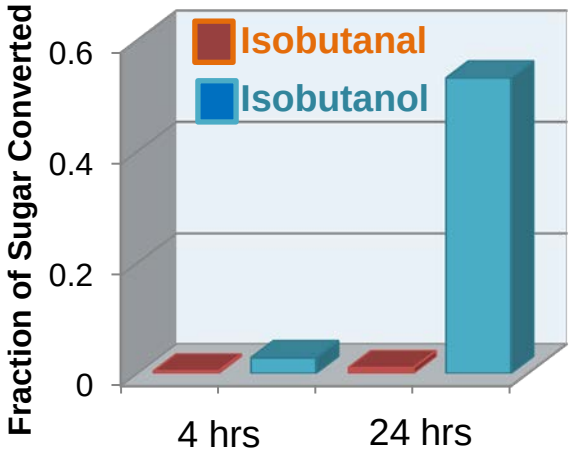
Analysis of other "polysaccharide hydrolysates" & *lignin streams* from other pretreatments

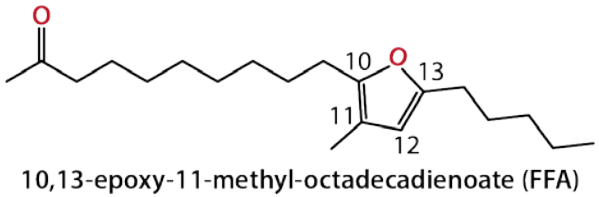
- γ-Valerolactone (GVL)
- Alkaline (alkaline hydrogen peroxide, extractive ammonia)
- Ionic liquids
- Monitor variations due to changes in enzyme cocktails



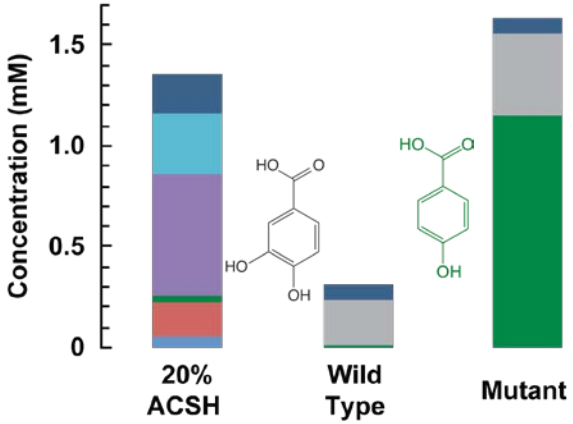


Systems Analysis of Biomass & Biofuel Production





10,13-epoxy-11-methyl-octadecadienoate (FFA)



Analysis of other microbial catalysts

- Additional fuels & chemicals (long chain alcohols, hydrocarbons, aromatics, etc.)
- Impact of changes on producing microbes
- Different single species/consortia

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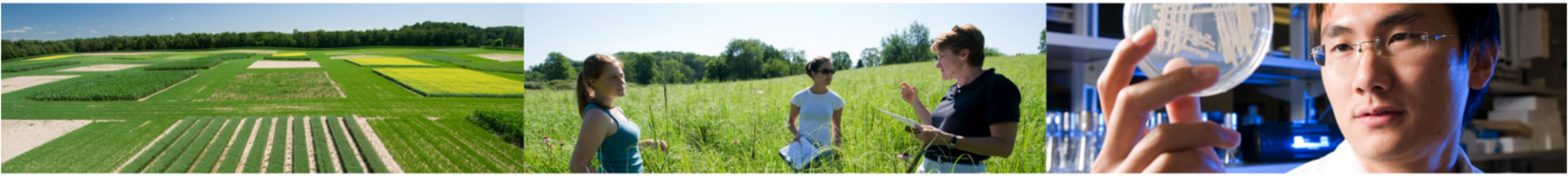

Questions?